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CONTENTS

PROCEEDINGS OF THE THIRTY-FIFTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE 133

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY .. 167

ESTUARINE ANIMALS AT BEAUFORT, NORTH CAROLINA. *A. S. Pearse* 174

RACIATION IN STENIRIDIA ANDREWSI HARRIS, A SUPPLEMENT TO SPECIATION IN STENIRIDIA. *J. M. Valentine* 223

NOTES ON THE HISTORY OF THE GERM CELLS IN THE TOADFISH (OPSANUS TAU). *Eugene P. Odum* 235

MORE PRIMITIVE MOSS-MITES OF NORTH CAROLINA. *Arthur Paul Jacot* 247

ORIBATA AND BELBA FROM FLORIDA (ACARINA, ORIBATINAE). *J. W. Wilson* 255

THE INCUBATION OF HEN EGGS UNDER INCREASED ATMOSPHERIC PRESSURE. *Bert Cunningham* 269

HETEROTHALLISM AND STERILITY IN ACHLYA AND OBSERVATIONS ON THE CYTOLOGY OF ACHLYA BISEXUALIS. *John Robert Raper* .. 274

A NEW SPECIES OF RHIPIDIUM FROM MOUNTAIN LAKE, VIRGINIA. *Velma D. Matthews* 291

THE GENUS CYPERUS IN NORTH CAROLINA. *Mildred Stites Reed* .. 295

SPERMATOGENESIS IN MARSILEA. *A. G. Lang* 307

CHAPEL HILL, N. C., U. S. A.

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Elisha Mitchell Scientific Society

Volume 52 December, 1936 No. 2

PROCEEDINGS OF THE THIRTY-FIFTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

DUKE UNIVERSITY, DURHAM, N. C., APRIL 24 AND 25, 1936

The thirty-fifth annual meeting of the North Carolina Academy of Science was held at Duke University, April 24 and 25, 1936. The meeting was called to order at 9:30 A.M. on April 24, by the president, W. L. Porter, after which the presentation of papers commenced and continued until 11:30 when the president announced the appointment of the following committees:

Auditing: T. E. Powell, P. M. Ginnings, D. B. Anderson;

Resolutions: B. W. Wells, H. E. Fulcher, L. A. Whitford;

Nominating: J. B. Derieux, Z. P. Metcalf, J. P. Givler.

At 1:00 P.M. the Academy took a recess for luncheon.

The reading of papers was resumed at 2:00 P.M. and continued until 3:45 when the Academy held its annual business meeting.

The minutes of the previous meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society.

Reports from the various committees were presented.

The executive committee, consisting of W. L. Porter, the president of the Academy, F. W. Sherwood, vice-president, H. L. Blomquist, secretary and treasurer, E. T. Browne, W. E. Speas, and H. R. Totten reported as follows:

"The executive committee met in Durham on April 24 and on April 25 with all members present.

"The committee appointed E. T. Browne to act as temporary assistant to the secretary during the meeting.

"Favorable action was taken on the request by Dr. William deB. MacNider that his paper be transferred to Friday afternoon.

"Two papers which arrived too late to go on the printed program were added to the program.

"The secretary-treasurer was authorized to purchase an interval-timer for the Academy.

"A special request made by Dr. Archibald Henderson and Dr. W. W. Elliott that Dr. Kasner of Columbia University be asked to speak twenty-five minutes before the Mathematics Section was granted.

"The committee voted to change the time of the business meeting from 4:30 to 3:45 P.M.

"The committee reported as elected to membership since the last meeting the following:

Mr. Harvey A. Bernhardt, Catawba College, Salisbury, N. C.
Miss Susan Blackwell, Catawba College, Salisbury, N. C.
Dr. Murray F. Buell, Dept. of Botany, N. C. State College, Raleigh, N. C.
Mr. Ed. H. Carter, Catawba College, Salisbury, N. C.
Mr. Joffre L. Coe, Box 652, Chapel Hill, N. C.
Mr. T. S. Coile, Dept. of Forestry, Duke Station, Durham, N. C.
Mr. James Doubles, Dept. of Botany, U. N. C., Chapel Hill, N. C.
Mr. Richard A. Edwards, Dept. of Geology, U. N. C., Chapel Hill, N. C.
Mr. John L. Etchells, Box 5323, Raleigh, N. C.
Miss Virginia Foil, Catawba College, Salisbury, N. C.
Dr. Coleen Fowler, Greensboro College, Greensboro, N. C.
Mr. M. E. Gardner, N. C. State College, Raleigh, N. C.
Dr. Joseph Greene, 331 Haywood Bldg., Asheville, N. C.
Mr. Clarence Harris, Route #2, Durham, N. C.
Dr. David H. Howard, Davidson College, Davidson, N. C.
Mr. V. A. Hoyle, Dept. of Mathematics, U. N. C., Chapel Hill, N. C.
Dr. Jack Levine, N. C. State College, Raleigh, N. C.
Dr. Alfred J. Maria, Box 4037, Duke Station, Durham, N. C.
Mr. Arthur C. Menius, Jr., Catawba College, Salisbury, N. C.
Miss Sarah M. Nooe, Queen's-Chicora College, Charlotte, N. C.
Mr. James R. Piland, N. C. State College, Raleigh, N. C.
Mr. Chilton E. Prouty, 1 Westwood, Chapel Hill, N. C.
Mr. George Rohde, Bausch & Lomb Optical Co., Washington, D. C.
Mr. Leland Shanor, Box 292, Chapel Hill, N. C.
Miss Rachel Smith, Catawba College, Salisbury, N. C.
Mr. T. E. Smith, Tobacco Exp. Station, Oxford, N. C.
Dr. Hertha Sponer, Dept. of Physics, Duke Station, Durham, N. C.
Dr. M. K. Veldhuis, Box 5042, Raleigh, N. C.
Dr. J. A. Wheeler, 416 Pittsboro St., Chapel Hill, N. C.
Mr. E. K. Whitner, Catawba College, Salisbury, N. C.
Mr. R. E. Wicker, Pinehurst, N. C.
Mr. H. C. Wilburn, Box 353, Waynesville, N. C.

"The following were reinstated to membership:

Miss Louise Adams, High Point College, High Point, N. C.
Dr. David Carpenter, Dept. of Physics, Duke Station, Durham, N. C.
Dr. F. W. Constant, Dept. of Physics, Duke Station, Durham, N. C.
Mr. Harry T. Davis, N. C. State Museum, Raleigh, N. C.
Dr. Archibald Henderson, Chapel Hill, N. C.
Dr. S. W. Hoffman, Statesville, N. C.
Prof. A. L. Hook, Elon College, N. C.
Dr. Charles W. Hooker, Duke Medical School, Durham, N. C.
Mr. W. E. Jordan, N. C. State College, Raleigh, N. C.
Mr. F. B. Meacham, N. C. State College, Raleigh, N. C.
Dr. J. C. Mouzon, Dept. of Physics, Duke Station, Durham, N. C.
Dr. Walter Nielsen, Dept. of Physics, Duke Station, Durham, N. C.
Dr. J. L. Stuckey, N. C. State College, Raleigh, N. C.
Mr. L. L. Williams, 223 McCauley St., Chapel Hill, N. C.
Dr. W. J. Wyatt, Wake Forest, N. C.

"The committee also reported the following losses during the year:

Lost by death:
Dr. T. G. Harbison,
Dr. Horace W. Frink.

Lost by resignation because of removal from state:
Dr. Eugene P. Deatrick.

Lost addresses:
Mr. Norman B. Foster,
Miss Dorothea R. McNutt,
Dr. Louise Perry.

Dropped from the roll because of non-payment of dues:
Twenty-four former members.

The Treasurer's report was as follows:

Financial Statement of the N. C. A. S. April 23, 1936

Receipts

Balance on hand May 1, 1935:		
Savings Account	$476.59	
Checking Account	73.43	
	$550.02	$550.02

Dues:			
1933	2.00		
1934	2.00		
1935	150.00		
1936	206.00		
Initiation fees:			
1935	28.00		
1936	42.00		
	430.00	430.00	
Overpay on dues		6.00	
Interest on savings		11.35	
N. C. Section Am. Chem. Soc. share toward programs—1935 and 1936		10.00	
Total Receipts ($26.00 transferred from savings to checking account)		$1007.37	$1007.37
Disbursements			
Stationery and printing	29.32		
Programs—1936	28.50		
Postage	1.77		
Refund on overpaid dues	6.00		
Books for H. S. Essay Prize	12.26		
Clerical Assistance	67.00		
Jour. of E. M. S. S.—1935. ($200.00 from checking and $24.00 from savings account)	224.00		
Charges on bank balance	.50		
Reprints of Proceedings and Constitution	15.60		
Express on Proceedings	.82		
Express on H. S. Essays	1.61		
Sec.-Treas. Commission	43.00		
Refund Sec. Dues	2.00		
Telegram	.47		
Receipt Book	.52		
Total Disbursements	433.37		433.37
Savings Account			
May 1, 1935	476.59		
Interest	11.35		
	487.94		
Withdrawn, Nov. 22, 1935 ($26.00 transferred to checking account; $24.00 to Journal)	50.00		
Balance April 23, 1936	437.94		

Total balance	574.00
In Savings Account	437.94
	136.06
Two dues on hand	4.00
Balance in checking account	132.06

The above report was made as of April 23, 1936.
Submitted by H. L. Blomquist, secretary-treasurer
Audited April 24, 1936 by
T. E. Powell,
P. M. Ginnings,
D. B. Anderson.

"The committee accepted the invitation of President Omwake of Catawba College to hold the thirty-sixth meeting in Salisbury.

"The executive committee made the following recommendations to the Academy:

1. That all bills presented in the treasurer's report be authorized and paid and that the report be printed when audited.

2. That Bert Cunningham be appointed to select the books to be presented to the winner of the High School Science Essay Prize, and that he be authorized to draw upon the treasury as much as $25.00 for these books; and that the secretary be authorized to appoint a representative of the Academy to award the prize and draw upon the treasury for the payment of his expenses.

3. That the discussion of each paper be limited to five minutes.

4. That the secretary be instructed when asking for titles to papers to ask, where two or more papers are presented for the general program by the same person, that the member indicate which paper should be placed first; also that the secretary state that it may be necessary if the program is crowded to place the second paper on a supplementary list to be called for if the time permits, otherwise to be read by title only.

5. That Dr. J. S. Holmes, state forester, of the State Department of Conservation and Development, and Dr. I. H. Manning of the Medical School of the University of North Carolina be made life members. Both of these members joined the Academy in 1902 and have continued their memberships up to date.

6. That the abstracts for the proceedings be limited to 250 words.

7. That for 1936 the treasurer be authorized to pay to the Elisha

Mitchell Scientific Society $300.00 instead of $1.00 per member as was done in 1935.

8. That a committee of three be appointed to work out plans by which the Academy may assist in financing the Journal of the Elisha Mitchell Scientific Society."

The auditing committee reported that they had examined the accounts of the treasurer for the period May 1, 1935, to April 23, 1936, and found them correct.

The reports of the treasurer and auditing committee were accepted.

The committee on high school science, consisting of Bert Cunningham, chairman, H. B. Arbuckle, C. E. Preston, Mrs. B. W. Wells, Lena Bullard, and Nancy Eliason, reported as follows:

"The committee reports that it has carried on the usual activities, namely, aiding in the district and state meetings of the science divisions of the North Carolina Educational Association, and the conducting of the contest for the High School Essay Prize.

"Your committee believes that a general survey of the high school science situation should be made, more especially as to courses offered, laboratory time allotted, equipment and materials provided, and the general quality of the work offered.

"It also believes that there should be a closer federation of high school science teachers and that such a federation should have an annual meeting either at the time of the Academy meeting or the state meeting of the N. C. E. A., for the purpose of discussing problems and exchanging ideas.

"The committee selected as judges for the essay contest, R. N. Wilson (Duke), chairman, Mary E. Petty (W. C. U. N. C.), and W. A. Speas (Wake Forest). There were 22 essays entered in the contest. The judges reported that Carl Deal of the Boyden High School, Salisbury, N. C., won the prize with an essay entitled "The Sea, a Mine."

"The committee recommends the continuation of the contest, the fields during the coming year to be biology or any of its subdivisions and geography."

The report of the high school science committee was accepted and its recommendations approved. It was voted that the committee be continued.

The legislative committee, consisting of Z. P. Metcalf, chairman, W. L. Poteat, Wm. F. Prouty, had no report at this time, but was instructed to continue.

The report of the elective appraisal committee, consisting of P. M.

Ginnings, chairman, E. H. Hall, R. E. Coker, Mary Conrad Cleaver, C. W. Edwards, Karl Fussler, E. G. Purdom, R. N. Wilson, J. T. Dobbins, was as follows:

"At the request of Catawba College, a sub-committee made a visit to this institution and as a result made the following recommendations:

"1. That the administration of Catawba College be informed of the constructive criticisms of the appraisal committee.

"2. That the departments in the sciences of Catawba College be approved for a period of three years subject to the absence of any radical changes."

The above report was accepted and the recommendations were approved by the Academy.

The conservation committee, consisting of Charles E. Raynal, chairman, B. W. Wells, J. P. Givler, C. F. Korstian, W. C. Coker, and J. S. Holmes, did not have a report but recommended that the committee be continued.

The recommendation of the conservation committee was approved.

The committee on public school curriculum, consisting of C. E. Preston, chairman, Mrs. B. W. Wells, and Lena Bullard, did not present a report.

A motion made by the chairman that the committee be discharged was passed.

The provisional committee on the A. A. A. S. grant, consisting of R. E. Coker, chairman, Milton L. Braun, C. F. Korstian, R. F. Poole, William L. Poteat, made the following report:

"The committee on awards appointed by the president early last winter was instructed (1) to nominate a candidate for the A. A. A. S. grant for the current year, and (2) to propose to the Academy a policy to be followed with reference to this award which is understood to be an annual grant.

"Your committee fulfilled the first function by recommending to the president and executive committee that the award for the current year be granted to Dr. Karl Z. Morgan of Lenoir-Rhyne College for research on 'Scattering of Cosmic Rays'. The decision was arrived at after very careful consideration of a number of applications received in response to a circular letter sent out from the office of the secretary of the Academy.

"In regard to the matter of future policy, the present committee recommends the election of a permanent committee of five, the first committee to be composed of two members elected for a term of one

year, two for a term of two years and one for a term of three years; thereafter, vacancies to be filled as terms of members expire (two members one year, two the next and one every third year) by the election of members to serve for a term of three years.

"The committee recommends that the regular nominating committee of the Academy shall at each annual meeting of the Academy submit nominations for membership on the permanent committee on awards.

"The present committee offers two suggestions:

"(1) That the selection of members of the committee be governed as far as is practicable by the principle of rotation among institutions and fields of research in science.

"(2) That the committee on awards give a reasonable degree of consideration to the relative needs of applicants."

This report of the committee on the A. A. A. S. grant was accepted and the recommendations were approved.

The following memorial reports honoring the late Dr. T. G. Harbison and Dr. Horace W. Frink were presented:

DR. THOMAS GRANT HARBISON

Thomas Grant Harbison died at Chapel Hill, N. C., on January 12, 1936. He was born in Forest Hills, Union County, Pennsylvania, the son of Thomas V. Harbison, April 23, 1862. He early showed remarkable mental ability and began teaching at the age of seventeen. He attended school during vacations, and being near Bucknell University he continued his studies under professors of that institution even while teaching. He never had a continuous year as a resident college student, work in vacations, the work under professors of Bucknell University, and later a short course in the University of Norway and one in the University of Leipsic completing that type of his training; but he early began to build up his own library, and by the time he was twenty-one he had a library of over a thousand volumes. This library he continued to add to throughout his life, and aided by a keen power of observation and a very retentive memory he truly educated himself. The education that he built was not a narrow one, and did not consist of mere reading at random. He applied himself to definite courses of study as laid out by the best correspondence schools of the time. In this way he followed through a four year course of study as outlined by the University of the City of New York, several years' study in the National University, the botanical part here under Dr. Coulter of the University of Chicago. By these methods he received through correspondence

courses the B.S., the A.M., and Ph.D. degrees and completed a course in Landscape Architecture and Gardening.

In 1886 young Harbison and a close friend, E. E. Magee, spent the late spring and the summer in an extended walking trip down into the south. This trip took them across Maryland and eastern Virginia to Norfolk, across North Carolina to Asheville, down to Highlands, N. C., near the Georgia line, back across North Carolina, up through the Shenandoah Valley of Virginia, across Maryland and home. From Caesar these two young men had learned that troops can march and fight on a ration of grain. They took as their equipment on this trip, in addition to their clothing, a small water proof bag to carry their ground wheat and some brown sugar, a tin bucket for a stove, and each had a copy of Wood's *Botany*. Except for the fruits along the way, they had practically no other food than the wheat mush sweetened with brown sugar, and yet they returned in fine condition. On one day, towards the end of the trip, they walked fifty-two miles. This trip had a great influence in Dr. Harbison's future life. It proved to him his own rugged constitution, and he never had fear of hard work, long hikes, and mountains; his first hand knowledge of and love for plants were increased; and he became convinced that in our own North Carolina mountains is a most satisfying place for the abode of a lover of nature. He had great love and respect for his native part of Pennsylvania, and through correspondence with relatives and friends and through a county paper[1] he kept in touch with it to the day of his death. However, this trip was the direct cause of his becoming a North Carolinian.

On his return to his home in Pennsylvania, he found that the people of Highlands had sent a call for him to teach their school. He returned to Highlands in the fall of 1886, and though away at brief intervals, he maintained a home there until death, practically 50 years later. He was the principal in public and private schools there from 1886 to 1893 and from 1895 to 1896. He developed there unique and effective methods of instruction. Naturally, plants had a great part in it. Besides reading, writing and arithmetic, the usual things learned in elementary schools, his pupils learned plants, not only their common names, but their Latin names as well, and what the Latin names meant and while doing so built a foundation for a knowledge of English. Through very small prizes, a penny for the first flower of each kind for

[1] The Lewisburg Saturday News. From the columns of this paper we have been able to secure a part of the information about him given in this article.

the season, five cents for the rare finds, he and they learned the flora of their region. And when funds ran low they kept their school going largely through shipping plants to northern markets. He became an advocate of an educational system that would provide a garden and work shop as a part of the elementary school.

The year 1893–94 Harbison traveled in Europe, studying the methods and results of such schools in Norway, Sweden, Denmark, Germany, and Switzerland. On his return he spent the following year lecturing on European schools and farming. For a time he edited a paper at Highlands. In 1896 he became principal of the Waynesville High School. One of the teachers in the school was his life-long friend and companion of his walking trip, E. E. Magee. The Waynesville High School was a private institution; but he set to work to have it taken over as a public school, and largely through his efforts it became one of the first public graded schools in the state. Also in 1896 he married Miss Jessamine M. Cobb, a daughter of Judd M. Cobb, the first building paper manufacturer in America, and a descendant of John Cobb, who came to Massachusetts in 1624 and built and operated the first iron foundry in America.[2]

The following year, 1897, he became a collector for the newly established Biltmore Herbarium of the estate of George W. Vanderbilt. The spring and early summer of 1898 he spent in studying and making collections of the plants in the eastern part of our state; the late summer and fall he spent in similar work in the Rockies, the Cascades, and the western Coast Ranges. He continued in such work, studying and collecting plants, mostly in our southern states, until the discontinuance of the herbarium at Biltmore in 1903. From 1905 to 1926 he was the southern representative for Dr. C. S. Sargent and the Arnold Arboretum, continuing the studying and collecting of southern flora especially the woody plants.

In 1929 he carried on botanical work for the Geological Survey of Mississippi. In 1933–34 his services were secured by the University of North Carolina to assist in assembling the Ashe Herbarium; and in 1934, at the age of seventy-two, at an age long after men of weaker build would have considered their work finished and well done, he was appointed Curator of the University of North Carolina Herbarium, the position he held at the time of his death. The University of North Carolina was most fortunate in securing his services for assembling

[2] In their Highlands home the Harbisons have the first iron crane made in America.

the Ashe Herbarium and directing its herbarium work. These two men, W. W. Ashe and T. G. Harbison, long friends and constant correspondents about the plants that they found in our southeastern states, and both prolific discoverers of new species, kept each other well informed of even the location of type plants from which the new species were described. These two, each with about a third of a century of field work, knew the woody plants of these southeastern states as no one else knew them. In his last illness, Mr. Ashe wrote Mr. Harbison, asking him in case of his death, to assist Mrs. Ashe in disposing of the Ashe Herbarium, and also expressed the hope that it could be secured by the University of North Carolina. It must be a considerable satisfaction to botanists everywhere that Mr. Harbison's own collection at Highlands has also since his death been secured by the University of North Carolina, and that the private collections of both of these men are to be found together in the same Herbarium, where they will continue to assist the present and later botanists in a way comparable to the assistance their collectors so freely gave to others while in life.

T. G. Harbison published the following botanical articles:

"New or Little Known Species of Trillium." *Biltmore Botanical Studies* 1: No. 1: 19. 1901, *Biltmore Botanical Studies* 1: No. 2: 158. 1902.

"A Sketch of Sand Mountain Flora." *Biltmore Botanical Studies* 1, No. 2. 151. 1902.

"Notes on the Genus Hydrangea." *American Midland Naturalist* 11: 255. 1928.

"Polycodium Ashei Harbisoni." *Midland Naturalist* 22: 179. 1930.

"Symplocos tinctoria Ashei, A New Dyebush from the Southern Mountains." *Journal of the Elisha Mitchell Scientific Society* 46: 218. 1931.

A Preliminary Check-List of the Ligneous Flora of the Highlands Region, North Carolina. *Highlands Museum and Biological Laboratory Publication* No. 3. 1931.

He had in manuscript a "Check List of the Woody Plants of Mississippi," results of field studies since 1915; also a "Check List of the Ligneous Flora of the Southern Appalachians," results of field studies since 1886.

The above list of publications hardly begins to measure the contributions of T. G. Harbison to the science of Botany. As a plant collector he recognized many new species and varieties of plants that he passed on to others for publication and he contributed greatly to the value of many publications through the gifts and loans of specimens and through his accurate memory as to the distribution of plants and the location of the types. His own herbarium is rich in type and co-type sheets.

He was very proud of the fact that he had visited nearly every type tree still living in the southeastern states, and the exact location of every type plant that he had ever seen seems to have been stamped indelibly upon his memory. We have been guided by him unerringly and with hardly any hesitation to type trees that he had not seen for thirty years. In recognition of his contributions to science the University of North Carolina chapter of the Sigma Xi elected him to active membership in 1935.

It is difficult to sum up briefly the life and contributions of this remarkable man. His work as a teacher in the schools of Pennsylvania and in the mountains of North Carolina was outstandingly successful and to the day of his death he was known and loved in our mountains as "Professor Harbison." His advocacy and influence helped to establish at Waynesville one of the first public graded schools in the state. In the same quiet way he was quite an influence in the establishment of the Western Carolina State Teacher's College at Cullowhee, N. C. He was an early advocate of manual training as part of the curriculum in each elementary school. As an active, influential member of the Junior Order and the Order of Masons he furthered the cause of education through those organizations. In his later years as a nature intructor in summer camps he continued the type of educational work that was nearest his heart. His public spirit was boundless. For a time he was mayor of his town; was for years road supervisor of his township and ever took pride in his part in the placement of some of the mountain roads. He was a pioneer in securing national forests for the western part of North Carolina, turned over much of his own land to the government at a very low rate and by his advocacy and example persuaded his neighbors to do the same. It should be mentioned here, however, that his last appearance before the North Carolina Academy of Science was in protest against a short-sighted National Forest policy of considering the purpose of National Forests almost solely that of raising timber trees and these of only a few kinds. Though a lover of timber trees, he also knew and loved all humbler plants too and fully realized their cultural value. He was an early advocate for a great National Park in the North Carolina mountains and was an early contributor and played a part in securing the coöperation of the mountain people in what has resulted in the Smoky Mountain National Park. He supervised the laying out and planting of a number of estates and through his knowledge of plants and their requirements, and through an innate sense of fitness and beauty he would open up distant vistas

and at the same time preserve the natural beauty of the native vegetation. As an orchardist he found and taught by experiment what varieties would be most successful in that apple region where North Carolina and South Carolina and Georgia meet. On his orchard farm on the southern slope of Mount Satulah he also conducted mountain tests as to the hardiness of new crops tried out or developed by the South Carolina Agricultural College and Experimental Station at Clemson, S. C. For years his family has kept the government weather records as to temperature and rainfall at the Highlands, N. C., station.

Harbison had a very influential part in the establishment and maintenance of the Highlands Library, the Highlands Museum, the Highlands Biological Laboratory. For years he gave freely his services as guide to practically every botanist and even to those only mildly interested in botany coming into the Highlands section. Up to within a year of his death when he was forced to give up strenuous mountain climbing by a severe attack of influenza that left his heart weakened, he could set a pace up mountain trails that any man half his age would have been proud to maintain. We have been on hard fifteen mile mountain hikes with him when he was past seventy years old. The memory of these mountain hikes and long rides, the botanical excursions through the mountains, the piedmont, the sandhills and coastal region of North Carolina, South Carolina, Georgia and Florida, the example of his keen and accurate observation, long talks at evening after the day in the field or herbarium (a memory and active life such as his made him a wonderful conversationalist), the patient help he gave to students makes us realize that in the passing of this cultured gentleman, scientist, we have lost a great teacher and friend. It was quite fitting that Thomas Grant Harbison should have spent his last days in the service of his state, in an honored position in its university. We wish that he could have carried on for years more the very valuable work he was doing and for which he was so well equipped. However, the thought of the loss is far outweighed by the thought of nearly seventy-four years of life as he lived it. While we send our sympathy to his wife, his three daughters, his son and his brother, still more we rejoice with them that Thomas Grant Harbison has lived.

H. R. TOTTEN,
W. C. COKER,
H. J. OOSTING,
Committee.

DR. HORACE WESTLAKE FRINK

Horace Westlake Frink was born at Irondale, N. Y., February 7, 1883. He died at a sanatorium near Southern Pines, N. C., April 18, 1936. His medical education was received at Cornell University Medical College, where he received the degree of Doctor of Medicine in 1905. Though first specializing as a surgeon, he early became interested in nervous and mental diseases, their psychological manifestations and therapy. For a time he studied under Sigmund Freud in Vienna, and he devoted the remainder of his life to work in these fields and attained eminence in them.

From 1914 to 1922 he was assistant-professor of neurology in Cornell University Medical College and was assistant attending neurologist at Bellevue Hospital, New York City. In 1922 he retired from teaching to devote himself to research and writing in psychology and psychiatry. He was the author of *Morbid Fears and Compulsions; Their Psychology and Psychoanalytic Treatment, Dreams and Neuroses,* and other titles. For a time he was associate editor of *The International Journal of Psychoanalysis.*

Among the learned societies to which he belonged are: the New York Neurological Society, the New York Academy of Medicine, the Neurological Association, the New York Psychoanalytic Society, of which he was president for three years, and the Psychopathic Association, of which he was secretary in 1915.

Having found in this region a location favorable to his not very vigorous health and congenial to his interests, he moved to Chapel Hill in 1932 where he subsequently bought a house and established his home. Coming to Chapel Hill without any connection with the University, he soon acquired a group of friends who found in him a man of worth and accomplishment and sincere genial manners. Though distinguished for modesty rather than assertiveness, his varied interests and talents brought him into contact with many phases of community and university life. Athletics and aesthetic crafts as well as scholarly and professional matters were subjects of eager interest to him. Though not wishing to build up a practice as a physician, his extensive knowledge and special talents in the field of psychiatry, coupled with his sincere desire to help others where he could, soon brought him into the relationship of friend and helpful adviser to many of those afflicted with or having to deal with nervous disorders. Though having no official connection with the University, he very generously and ca-

pably served as occasional special lecturer to the classes in neuro-anatomy of the Medical School and to the students in the Department of Psychology.

It was typical of his interests and coöperative nature that shortly after coming to the state Dr. Frink affiliated himself with the North Carolina Academy of Science and took an active interest in both the social and scientific aspects of its sessions. In his death the Academy has suffered the loss of a valued and distinguished member. To his son and daughter, his widow, and other members of his family, the Academy wishes to extend its deepest sympathy in their grief.

J. B. BULLITT,
I. H. MANNING,
W. C. GEORGE,
Committee.

The general resolutions committee, consisting of B. W. Wells, chairman, H. E. Fulcher, and L. A. Whitford, reported as follows:

"It is with great regret that we record the recent deaths of Dr. Horace W. Frink, Dr. Julia Dale, and Dr. Charles F. Meserve, former president of Shaw University.

"Dr. Frink was a psychoanalyst formerly on the faculty of Cornell University. He resided in Chapel Hill. He died at the age of 53.

"Dr. Julia Dale, for several years assistant professor of mathematics at Duke University, was at one time an active member of the Academy.

"President Meserve was for many years an active member of the Academy. Though his major field of activity was not that of science, he was profoundly interested in biological science and geology. In his later years he was an active member of the Raleigh Natural History Club. He died at the age of 86.

"Through your resolutions committee the North Carolina Academy of Science desires to express its great appreciation of the manner in which Duke University has entertained North Carolina's leading science organization. The opportunities to visit the great Duke Hospital and the extensive Duke Forest proved to be especially instructive experiences. The tea, dinner, and organ recital, so generously given to the Academy contributed to the social side of the meeting and were much appreciated. We give voice to the feeling of every member attending when we state that the Academy is deeply grateful for all the courtesies shown by its host, the faculty of Duke University, under the chairmanship of Dr. Bert Cunningham."

Bert Cunningham, representative of the Academy to the A. A. A. S., reported as follows:

"The Academy Conference for 1935–36 was largely devoted to the problem of Junior Academies, with some injection of the problems of branches of the A. A. A. S.

"The session was opened with a History of the Accomplishments of the Academy Conference by H. E. Enders. Secretary Bilsing rapidly sketched some of the things which the Academy Conference might do. In a later conference your representative found that other academies were not much interested in the publication of Academy programs in Science.

"Watson Davis pointed out that a publication was desirable for the Junior Academies and suggested devoting a portion of the Science News Letter to this service. A rather extended report was made on coördination of science clubs, and source material for them.

"The increase in high school science clubs has made it desirable to have them affiliated with each other in order to make possible a more satisfactory method of securing and distributing source materials, programs, and working ideas. Since the move is supposedly scientific it seemed desirable to have the various groups under the eye of some scientific organization. The State Academy seemed to be the most available and the most satisfactory foster parent—especially if some one in the Academy would assume the directorship of such an organization.

"A conference with Dr. Caldwell, chief proponent for the clubs, led to clarification of the situation, both from his standpoint and from ours. It was pointed out to Dr. Caldwell, and he concurred, that the high school committee of our Academy in its present functioning was probably serving the function of director; that the loose association which the Academy maintains with science teachers in the secondary schools is satisfactory in a diffuse population such as occurs in North Carolina; that a federation of clubs might for the same reason be undesirable and that for the "federated" clubs to meet at the time and place of the Academy would be impractical.

"In conferences with Dr. Ward we were commended upon our high school prize plan, and were asked by him to submit details of our plan. It was made clear by both that no pressure would be brought to bear for a Junior Academy in this state.

"Your representative concurs with the Academy conference that a publication devoted to high school science and high school science clubs would be highly desirable, but doubts the advisability of diverting a part of the Science News Letter to that end.

"The report on research grants indicated that many of the academies accepted and approved of the change. It was quite apparent from the general secretary's financial report to the A. A. A. S. that the change was imperative. The funds for current expenses (from which the Academy rebates were made) have for several years fallen short of expenditures. On the other hand the Association has a fund which can be expended only for research, and it is from this fund that the grants are now made. Assurance was given that similar grants would be made for the coming year. Your representative agrees that the present procedure is justified under the circumstances.

"The problem of local branches of the A. A. A. S. was mentioned here as well as in the Council meetings. To your representative it seems that a local branch would in no way interfere with the Academy. The branch has a more or less popular meeting and is not so much interested in new contributions, as such, as in the stimulation of scientific interest and the dissemination of scientific knowledge. Membership is not restricted to members of the A. A. A. S., and the move is not to increase membership in that organization. It is desirable from the standpoint of the A. A. A. S. that the Academy sponsor and coöperate with local branches.

"The time remaining for the discussion of other problems was insufficient and informal discussion was carried on at the dinner.

"It seems to your representative that little of the primary purpose of the Academy Conference has been achieved during the several years of its existence, and it is to be hoped, that Junior Academies and A. A. A. S. branches will give way to more thoughtful discussion of how the academies may improve themselves by profiting by the experience of others.

"While for the most part the Council meetings were devoted to routine affairs there were some items of especial interest to this Academy. Two of these are dealt with in the report on the Academy Conference. Another was the establishment of a Southeastern Division of the A. A. A. S. Your representative pointed out that North Carolina being a "border state," would most likely prefer to meet with the A. A. A. S. if its meetings were within range, and that a meeting in the Southeast will probably be feasible, so far as this state is concerned, only when the general meeting is far distant.

"The Georgia Academy invited representatives of the various academies of the Southeast to meet with them at the time of their annual meeting and discuss the problem. Representatives of the A. A. A. S. were also invited. Although the meeting has been held your representative does not know what action, if any, was taken.

"It appears to your representative that it would be more feasible to have a loose federation of Academies of the Southeast which could have a Christmas meeting when the A. A. A. S. meets at some distant point. Such meeting should be centrally located and planned for several years in advance. Since the next two meetings are to be held at Atlantic City and Richmond, it is doubtful if any meeting should be held within the next two years."

The nominating committee submitted the following nominations:

President: P. M. Ginnings, Greensboro College;

Vice-President: C. F. Korstian, Duke University;

Secretary-Treasurer (for three years): H. L. Blomquist, Duke University;

New Member of the Executive Committee (for three years): W. L. Porter, Davidson College.

Committee on A. A. A. S. grant:

R. E. Coker (3 years), chairman,
M. L. Braun (2 years),
O. J. Thies (2 years),
J. G. Boomhour (1 year),
Eva G. Campbell (1 year).

The nominations were accepted and the secretary was instructed to cast the ballot of the Academy for the nominees.

The president then announced the appointment of the committee to assist the Elisha Mitchell Scientific Society to finance the Journal, as follows: Z. P. Metcalf, H. R. Totten, and H. L. Blomquist.

The business meeting then adjourned.

At 8:00 P.M. the members of the Academy were entertained at a complimentary dinner given by Duke University in the Union reception room.

At the conclusion of the dinner, with F. W. Sherwood, vice-president presiding, an address of welcome was made by W. C. Davison, Dean of the Duke University Medical School. This was followed by the presidential address, entitled "The Teacher of Science" by the retiring president, W. L. Porter.

On Saturday morning the Academy met in sections. President Porter presided over the General Section; W. N. Mebane, Jr., over the Mathematics Section; C. C. Hatley, over Physics Section; and R. W. Bost, over the Chemistry Section.

The following officers were elected by the respective sections:

Mathematics Section—Chairman, J. H. Roberts, Duke University; Secretary, J. M. Clarkson, North Carolina State College.

Physics Section—Chairman, M. L. Braun, Catawba College; Secretary, J. S. Meares, North Carolina State College.

North Carolina Section of the American Chemical Society—Chairman, Edward Mack, Jr., The University of North Carolina; Vice-chairman, W. C. Vosburgh, Duke University; Secretary-treasurer, E. C. Markham, The University of North Carolina; Councilor, L. A. Bigelow, Duke University; Executive Committee, the officers, R. W. Bost, The University of North Carolina (3 years): C. S. Black, Wake Forest College (2 years); W. A. Reid, North Carolina State College (1 year).

The following papers were presented. Those marked with * appear in full in this issue. Those marked x are abstracted with the Proceedings. Those marked † were read by title.

GENERAL SECTION

**The Effects of Increased Atmospheric Pressure on the Developmental Rate of Chick Embryos* (Final Report) (Lantern). BERT CUNNINGHAM, Duke.

x*Faunal Statistics of North Carolina.* C. S. BRIMLEY, N. C. Dept. Agr.

x*The Effect of Periodic Illumination upon Cell Wall Structure* (Lantern). DONALD B. ANDERSON, State.

Stimulation of Seedling Plants by Organic Matter (Lantern). J. R. PILAND and L. G. WILLIS, N. C. Agr. Exp. Sta.

x*Treatment of Coastal Plain Waters for Domestic and for Industrial Users.* E. E. RANDOLPH, State.

Some of the Rarer Orchids of North Carolina. DONOVAN S. CORRELL, Duke.

x*A New Phosphorescent Species of the Genus Achromobacter.* FRANK H. JOHNSON and IVAN V. SHUNK, State.

New and Little Known Algae from North Carolina. L. A. WHITFORD, State.

Paper appeared in full in this Journal **52**: 93. 1936.

x*Morphology and Hormone Production by the Testis in Relation to Age* (Lantern). C. W. HOOKER, Duke.

The Vegetation of a "bare-faced" Cliff in Western North Carolina (Lantern). HENRY J. OOSTING and LEWIS E. ANDERSON, Duke.

Physiological Studies at High Altitudes (Lantern). F. G. HALL, Duke.

An Electro-mechanical Device for Determining Humidity under Pressure (Demonstration in Room 223). WILLIAM HURST and BERT CUNNINGHAM, Duke.

The Effect of Sodium, Potassium, and Calcium Ions on Permeability of Amoeba proteus to water. COLEEN FOWLER, Greensboro College.

x*Changes in the Vegetation of the Forest Floor following Elimination of Root Competition* (Lantern). C. F. KORSTIAN, Duke.

†*Thermal Zones in the Carolina Mountains.* MARTHA E. NORBURN, Biltmore.

x*Inactivation of the Virus of Common Tobacco Mosaic in Soil* (Lantern). S. G. LEHMAN, State.

The Acquired Resistance of Fixed Tissue Cells (Lantern). WILLIAM DEB. MACNIDER, U. N. C.

Resistance of the Air to (a) a Base Ball, (b) a Tennis Ball (Opaque Lantern). J. B. DERIEUX, State.

Carolina Bays and Current Explanations (Opaque Lantern). WILLIAM F. PROUTY, U. N. C.

x*The Osmotic and Porous Properties of Oil Films.* CHARLES M. HECK, State.

Old Age Characteristics of the Ostracod Genus Ctenobolbina (Opaque Lantern). RICHARD A. EDWARDS, U. N. C.

x*The Susceptibility of Stretched Rubber to Temperature Changes.* MILTON L. BRAUN, Catawba.

The Paleontologist's View of Species. J. W. HUDDLE, U. N. C.

x*The Relation between Rate of Transpiration and Rate of Water Absorption in Plants* (Lantern). PAUL J. KRAMER, Duke.

Origin of Southern Appalachian Grass Balds (Lantern). B. W. WELLS, State.

x*Auto-narcosis in the Oyster and its Industrial Application.* HERBERT F. PRYTHERCH, U. S. Fisheries, Beaufort.

x*Composition of the Leaf Litter of Forest Trees* (Lantern). T. S. COILE, Duke.

The Soil Factor in Crop Adjustment. J. F. LUTZ, State.

Use of Trisomics in Linkage Studies. H. S. PERRY, Duke.

x*The Embryo Sac of Acorus Calamus L.* (Lantern). MURRAY F. BUELL, State.

Peat Mosses (Sphagna) of North Carolina. H. L. BLOMQUIST, Duke.

Chromosomes in the Germ Cells of Epilachna corrupta Mulsant. H. M. PHILLIPS and O. C. BRADBURY, Wake Forest.

Production of mature Perithecia of Cordyceps militaris Link in Culture. LELAND SHANOR, U. N. C.

Paper appeared in full in this Journal **52**: 99. 1936.

Water Absorption by Reptile Eggs during Incubation (Lantern). BERT CUNNINGHAM and A. P. HURWITZ, Duke.

The Effects of Endocrine Feeding of Flesh-Fly Larvae (Lantern). E. C. HESTER and BERT CUNNINGHAM, Duke.

The Tracing of Basic Dikes by Electrical and Magnetic Geophysical Methods. W. R. JOHNSON, JR., G. R. MACCARTHY, J. C. MACCAMPBELL, and H. W. STRALEY III, U. N. C.

Some Ostracods from the Sneedville Limestone of Eastern Tennessee. HOWARD E. VITZ, U. N. C.

MATHEMATICS SECTION

x*Geometricl Transformations.* EDWARD KASNER, Columbia University.

x*Some Remarks on Stieltjes Integrals.* F. G. DRESSEL, Duke.

x*Some Theorems on Tensor Differential Invariants.* JACK LEVINE, State.

x*On the Equation of Conics of r-point Contact.* J. W. LASLEY, JR., U. N. C.

Equilibrium Point of Green's Function for a Spherical Shell. A. J. MARIA, Duke.

x*Algebraic Systems.* J. M. THOMAS, Duke.

PHYSICS SECTION

x*The Effects of Acids and Bases upon the Infra-red Absorption Bands of Water* (Lantern). E. K. PLYLER and E. S. BARR, U. N. C.

x*Heat Transfer across an Interface between Oil and Water.* C. M. HECK and GRADY W. BARTLETT, State.

Resistance of the Air to (a) a Basket Ball, (b) a Foot Ball, sidewise and endwise (Opaque Lantern). J. B. DERIEUX, State.

Radioactive Fluctuations. A. E. RUARK, U. N. C.

Infra-red Absorption of HCl in Benzene. DUDLEY WILLIAMS and E. K. PLYLER, U. N. C.

On Photochemical Processes in Solutions. H. SPONER, Duke.

An Attempt to Obtain a Proton Source by Diffusion through Palladium. S. T. MARTIN and F. W. CONSTANT, Duke.

Infra-red Absorption of Phenol and Alcohol. J. W. WHITE and E. K. PLYLER, U. N. C.

Fine Structure in Nuclear Disintegrations. J. A. WHEELER, U. N. C.

The Design and Control of a High Current Mercury Arc. R. T. DICKERSON and W. M. NIELSEN, Duke.

Investigations on the Operation of a Van de Graaff Generator. C. H. TOWNES and F. W. CONSTANT, Duke.

Shower Production in Lead and Other Elements. J. E. MORGAN and W. M. NIELSEN, Duke.

The Absorption of Cosmic Ray Shower Particles in Lead and Other Elements. J. E. MORGAN and W. M. NIELSEN, Duke.

A New Voltage Regulator Circuit. J. A. ASHWORTH and J. C. MOUZON, Duke.

A Simple Method of Discriminating and Measuring Multiple Coincidences in Geiger Müller Counters. J. C. MOUZON, Duke.

EXHIBITS

Luminous Bacteria of the Genus Achromobacter. FRANK H. JOHNSON and IVAN V. SHUNK, State.

Morphology and Hormone Production by the Testis in Relation to Age. C. W. HOOKER, Duke.

Vegetation of North Carolina in Photographs. H. L. BLOMQUIST, Duke.

Peat Mosses of North Carolina. H. L. BLOMQUIST, Duke.

NORTH CAROLINA SECTION OF THE AMERICAN CHEMICAL SOCIETY

The Zinc Electrode. WILLIAM C. CLAYTON and WARREN C. VOSBURGH, Duke.

The Determination of Small Amounts of Alcohol and Water in U. S. P. Ether. F. H. SMITH, State.

Aldoximes and their Acyl Derivatives. C. R. HAUSER and E. JORDAN, Duke.

Double Sulfates containing Ferric Sulfate. FRANK K. CAMERON, U. N. C.

Partial Pressure of Hydrogen Chloride from Benzine Solutions. J. H. SAYLOR, Duke.

Business Meeting.

Oils and Waxes in Whole Cotton Plant. A. R. MACORMAC and DAVID MILNE, U. N. C.

The Accuracy of the Vitamin B. Assay. F. W. SHERWOOD, State.

The following abstracts have been received:

Faunal Statistics of North Carolina. C. S. BRIMLEY.

Summarises the fauna of the state as represented on the cards of the Division of Entomology of the State Department of Agriculture as follows:—

Total forms recorded, 11,560; of which 977 are vertebrates and 10,583 invertebrates.

The vertebrates include 90 mammals, 370 birds, 72 reptiles (including 1 crocodilian, 42 snakes, 9 lizards, and 20 turtles), 65 amphibians (of which 27 are frogs and 38 salamanders), and 380 fishes.

The invertebrates include 8 protochordates, 9,754 arthropods (of which 9,057 are insects, 368 arachnids, 265 crustaceans, and 64 myri-

apods), 197 worms, of all groups, 378 mollusks, 24 echinoderms, 88 coelenterates and sponges, and 134 protozoans.

The insect total of 9,057 is divided among 23 orders, of which the most important are Coleoptera with 2,919, Diptera, 1,638, Hymenoptera, 1,502, Lepidoptera, 1,070, Homoptera, 590, Hemiptera, 497, Orthoptera, 220, Odonata, 126, Mallophaga 119, the other 14 orders containing less than a hundred species each on record. The smallest order however, the Isoptera, containing the termites is in spite of having only three North Carolina representatives one of the most important economically.

The Effect of Periodic Illumination upon Cell Wall Structure. D. B. ANDERSON.

Potato plants were grown from a single tuber under conditions of complete darkness, constant illumination, and normal day and night. The collenchyma cells of the stem showed the same characteristic lamellation under each of these conditions. The variation in exposure to light produced no change in the structure of these cell walls.

Cotton fibers were grown under constant artificial illumination and under normal day and night conditions. Cross sections of the fibers showed no apparent differences in wall thickness. When swollen and subjected to pressure the fiber produced under outdoor conditions showed a striking lamellation. Fibers grown under constant artificial light showed no lamellation.

The wall of the cotton fiber is therefore fundamentally different in its structure and in its response to variations in environmental conditions from some other cell wall types.

Treatment of Coastal Plain Waters for Domestic and for Industrial Uses. E. E. RANDOLPH.

Surface waters of western North Carolina are as pure as any in the United States. Consequently many important industries with exacting requirements for quality of water are located in this area. The rivers rising in the mountains and flowing eastward become more highly charged with mineral and suspended matter as they approach the sea. The types of industries of the coastal plain consequently become somewhat limited. Over a period of years this investigation has been in progress to find feasible means to treat the various water supplies to make them entirely suitable for domestic and for practically all types of industries.

By inducing a larger number of wholesome industries to locate in

this area more people would be given employment and the abundant natural resources could be converted into useful products of economic value and a greater number of weekly payrolls would result in placing more money into circulation.

Much of the water of the coastal plain is suitable for certain types of industries by ordinary filtration, and by simple treatment would meet the more exacting requirements of other industries. However some of the surface and well water supplies are more difficult to condition for altogether satisfactory domestic, boiler, laundry, and special processing uses. The chemical engineer finds some real problems in some of the water supplies, but most of these problems can be economically met. The prevailing excess mineral matter consists chiefly of salts of Ca, Mg, Fe, and Na, and in some cases Mn, associated largely with bicarbonates and chlorides, with some phosphates, sulphates, and hydrogen sulphide.

Because of the abundant vegetable growth some of the surface waters contain considerable organic matter including tannic and other acids. Some of the waters are highly colored and sometimes have unpleasant odor and taste.

Near the coast sodium salts from the tides and from deep wells present serious problems.

Methods suggested for overcoming these difficulties are successive flocculation with ferrous sulphate at suitable alkalinity, accompanied by chlorination to remove color and aeration and activated carbon to remove odor and taste. To remove iron raise pH, aerate by spraying and distributing the water to present all possible surface by trickling in droplets over trays, coke, or by other means. Let settle at least four or five hours and filter.

Manganese may be removed in manner similar to iron except that pH should be higher, accompanied by chlorination and by much longer coagulation and settling period.

Since calcium and magnesium exist mainly as bicarbonates and it is desired to keep sodium salts to a minimum, lime carefully proportioned will reduce hardness to suitable limits if the water is previously aerated to remove carbon dioxide.

To overcome the brine problem from tides and from deep wells it is found that shallow wells can be sunk to yield comparatively fresh water by observing in regard to the depth the fundamental relations concerning diffusion of salt ground waters with fresh ground water near the sea coast.

The details of design and operation of any of these operations is a

major chemical engineering problem. With proper planning and operation, suitable and economic supplies of water should be possible for domestic and exacting industrial requirements.

A New Phosphorescent Species of the Genus Achromobacter. FRANK H. JOHNSON AND I. V. SHUNK.

A new species of luminous bacteria was isolated by the senior author from a dead amphipod (*Telorchestia* sp.) at Woods Hole, Mass., during the summer of 1935. It is morphologically very similar to *Achromobacter Fischeri* but may be readily distinguished from it, as well as from many other species of luminous bacteria, by virtue of the fact that neither its growth nor luminescence is favored by glycerin, and it produces no acid from this substrate in culture. Other definite physiological and cultural characteristics separate it from all reasonably adequately described species reported in the literature. The name of *Achromobacter Harveyi* has been proposed for this species.

Morphology and Hormone Production of the Testis in Relation to Age. CHARLES W. HOOKER.

In a series of 35 bulls ranging in age from one month to fifteen years the testes of each animal and a 24-hour urine specimen from each animal have been extracted for the "male sex hormone." A small portion of each of the testes was studied histologically. Assay of the testis extracts showed the hormone, present at all ages, to increase gradually, in general, to shortly after two years of age when there is a marked increase reaching a maximum at five years, after which the quantity of hormone present decreases progressively. Since the changes accompanying puberty occur in the absence of a concomitant increase in sex hormone production, the hypothesis is advanced that at puberty the tissues acquire a capacity to respond to an amount of hormone to which these tissues had previously been refractory. The diminution of hormone production which was found in the old animals indicates that senile changes may perhaps indeed be due to a withdrawal of the hormone. In marked contrast to human urine, in which the hormone is present, the hormone was absent from all these bull urines.

Histologically the young calf testis shows intertubular spaces occupied almost exclusively by mesenchymatous cells. These cells have two possible fates: connective tissue, or Leydig cells. Accompanying the completion of the development of the Leydig cells there is a marked rise in hormone production. As these Leydig cells increase in number

hormone production increases; and as these cells decrease in number in the older animals and are replaced by connective tissue, hormone production decreases. The correlation between the development of the Leydig cells and hormone production provides strong evidence that these cells are the site of production of the hormone in the testis.

Changes in the Vegetation of the Forest Floor following Elimination of Root Competition. C. F. KORSTIAN.

Whenever trees grow close enough together to form a forest stand competition occurs, that is there is a struggle between the individuals for growing space, both above and below ground, and for light, nutrients, and water. Competition results in the overtopping and crowding out of the weaker trees by the more vigorous and more aggressive ones, resulting in a natural "survival of the fittest." A tremendous struggle occurs in forests growing in moist to wet situations as they develop from very dense young stands composed of many small trees to mature forests made up of a much smaller number of large trees.

The study of the competition between the trees in a forest stand has been under way in the Duke Forest for several years. One phase of this study has involved repeated observations on 15 small areas in five different types of forest to determine the changes which take place in the vegetation of the forest floor following the elimination of root competition. In seven of the areas under observation all tree roots extending into the soil of the areas from the outside were severed by digging a trench 3 to 4 feet deep around the areas, thus eliminating the competition of tree roots for moisture and nutrients. The trenches were filled up again and detailed maps were made showing the location of all plants on each area. At least once each year thereafter a new set of maps was made. Similar maps were made for nearby untrenched areas. In this way it has been possible to follow the detailed changes in the vegetation which took place on the areas. The trenched areas have revealed very striking and almost unbelievable changes in the plant cover of the forest floor in two to three years. A great many plants have sprung up making the vegetation much more dense on the trenched than on the untrenched areas. Many additional species of plants commonly inhabiting moist areas have appeared. On many of the trenched areas, especially on the drier ones, the vegetation has become so dense and luxuriant that competition on the inside of the trenches will doubtless become intense as the tree seedlings develop. When this occurs the amount of soil moisture in the trenched areas will

probably decrease. However, determinations of soil moisture, made at intervals throughout each growing season since this experiment was begun, have thus far showed that the trenched areas contained a significantly greater amount of soil moisture than their corresponding untrenched areas every growing season since establishment. Light intensities were practically the same on the paired trenched and untrenched areas.

Evidence now available points to a very material hastening of the invasion of the trenched pine areas by hardwood trees, which commonly occur in the oak-hickory or bottomland forests of the piedmont region, due to the greater amount of soil moisture available in the trenched than in the untrenched areas. This study provides further evidence that soil moisture may often be more important than light in the death of trees in dense forests.

Inactivation of the virus of common tobacco mosaic by drying and by freezing in soil. S. G. LEHMAN.

When a field bears a crop of tobacco diseased with mosaic, the soil becomes contaminated with the virus of mosaic, but there appears to be no progressive accumulation of virus in soil with production of several successive diseased crops. The virus in the soil may be incorporated in diseased leaf, stem, or root material or it may leach from the decaying plant tissue and exist as virus particles in intimate contact with soil particles. The presence of small quantities of virus in soil may be demonstrated by appropriate methods.

When soil containing virus particles is allowed to become air dry, all or a considerable portion of the virus is inactivated and does not become active again when water is added. In drying soils which contain considerable amounts of virus, all or a large part of the virus is inactivated by the time the water content of the soil has been reduced to 4 to 5 per cent of its water-holding capacity. Freezing and thawing also inactivates tobacco mosaic virus in contact with soil particles, but is less effective quantitatively than drying.

Drying and freezing are factors which tend to prevent great accumulation of mosaic virus in tobacco soils .

The Osmotic and Porous Properties of Oil Films. C. M. HECK.

Continuing the measurement reported at the 1935 meeting of the Academy of the rate of evaporation of water through thin layers of oil in freely circulating air, measurements were made in closed rooms in

which the thermograph and humidigraph showed twenty-four hour variations of less than 2°C. and 4% relative humidity. The graph of evaporation rate plotted against the thickness of oil film with thirty evaporating pans gave the same type of semiperiodic rising and falling curves that were formerly obtained in more rapidly circulating air. The conclusions were drawn that oil films in spreading tend to form into two or more types of films in which the molecular organization tends toward one form more than the other as it approaches certain thicknesses of the oil film and that the difference in the porosity due to the amount or type of organization of the molecules of the film was more effective in producing porosity than the difference in thickness.

The formerly reported discovery of the osmotic properties of thin films of mineral oils was followed up by more extended research into the method of making and testing these osmotic oil films. After soaking porous earthenware vessels in oil, drying for a few days, soaking again in gasolene, redrying, and filling with sugar solutions, the osmotic force driving water into these solutions through the vessels was found to rise in a few hours to as much as ten centimeters of mercury and fall gradually in as many days to nothing.

The Susceptibility of Stretched Rubber to Temperature Changes. MILTON L. BRAUN.

Ring-shaped rubber bands, 1 x 4 mm. in cross-section and 10 cm. in diameter, vulcanized in molds at the National Bureau of Standards, were subjected to various gravitational stresses in a controlled temperature chamber for more than 500 hours. At scattered times the temperature of the chamber was slowly raised or lowered several degrees and the resulting lengths of the bands were observed. With loads above a certain modest value the historically determined decrease in length with increase in temperature was confirmed, while for very low stresses the contrary effect of extension with increase in temperature also was confirmed. One of the chosen loads happened to lie very close to the critical value for that particular compound, and for this load, throughout temperature ranges of nearly twenty degrees, there were no observable changes in length. Most of the specimens, under their respective stresses, showed constant rates of change in length with temperature, this being true even after three weeks of drift. These constant rates for different loads were found to range from plus .00171 to minus .0550 cm. per degree centigrade. The corresponding stretches ranged from negligible to

about five times the original length of the band, the stretches being produced by loads between 10 and 1000 grams.

An appreciable time lag between the response of the bands and the response of adjacent mercury-in-glass thermometers also was observed. With one thermometer the lag behind the response of the rubber was more than a minute. Stretched rubber therefore seems to be more susceptible to temperature fluctuations than is the more sluggish mercury or alcohol thermometer, and it is conceivable that under certain local conditions a thermoscope or thermometer actuated by a piece of rubber under tension might be more serviceable than the conventional type of thermometer.

The Relation between Rate of Transpiration and Rate of Water Absorption in Plants. PAUL J. KRAMER.

The most important factor in plant water relations is really the internal water balance of the plant. This in turn is governed by the relative rates of absorption and transpiration, and the ratio of transpiration to absorption is therefore really more important than the absolute rates of the processes. Few simultaneous studies of these processes have been made because of the difficulty of measuring the absorption of plants rooted in soil. A series of such measurements were made by supplying the plant with water through Livingston auto-irrigators which permit measuring the rate of absorption of plant-plus-soil and simultaneously measuring the loss of water by weighing. The rates of absorption and transpiration were measured simultaneously at two-hour intervals over a 24-hour period for green ash, loblolly pine, sunflower, and *Opuntia*. In all four species transpiration exceeded absorption from early morning until late afternoon while absorption exceeded transpiration during the night. The maximum rate of transpiration occurred in the period from 12 to 2 p.m. in ash, pine, and sunflower, and from 4 to 6 p.m. in *Opuntia*. The maximum rate of absorption occurred two to four hours later.

The results of this experiment seem to indicate that during times of moderate or rapid transpiration plants cannot absorb water as rapidly as they lose it and a considerable saturation deficit therefore usually exists in transpiring plants during the day and even much of the night. This probably has important retarding effects on the physiological processes associated with growth. Under conditions favoring rapid transpiration and slow absorption, the amount of water absorbed at night may not equal the amount lost during the day for several days.

Such a condition results in cessation of growth and if too long continued causes death.

Auto-Narcosis in the Oyster and Its Industrial Application. H. F. PRYTHERCH.

In very young stages of *Ostrea virginica* the functioning of many internal organs can be clearly seen through the transparent shell. Soon after shell closure there is complete cessation of heart action, and movements of the blood, cilia, and digestive tract, which is accompanied by a rapid increase in the hydrogen-ion concentration of the liquor. During the first 15 minutes after closure the pH of the liquor changes from an average of 8.1 to 6.7, and gradually drops to 6.2 in oysters kept out of water for 3 to 5 days.

Experiments have shown that the tissues of the oyster are narcotized, after the shell closes, by the CO_2 generated principally by the gills, and that this condition is due primarily to the rapid development of a high CO_2 tension within the shell rather than to an increased H-ion concentration. Tests with various organic and inorganic acids show that complete narcosis cannot be produced at the pH values stated above. Carbonic acid is the exception to the rule and is particularly suitable for conserving the energy and prolonging the life of the oyster outside of its natural environment, as it readily penetrates and leaves the tissues without producing injury or noticeable after effects, as is the case with most narcotizing substances.

In commercial operations approximately 60 per cent of the oysters are shucked and wasted before marketing. It was found that the meats were rarely killed by shucking and would survive washing in fresh water up to three minutes. If then placed in the usual air-tight tin containers they would generate sufficient CO_2 to produce narcosis and reach the market in a fresh and live condition as if shipped in the shell. A small amount of "Dry Ice" or gaseous carbon dioxide can be introduced if necessary into the containers to improve the keeping qualities of the meats.

Composition of the Leaf Litter of Forest Trees. T. S. COILE.

The relatively undecomposed organic debris which characterizes the uppermost layer of the undisturbed forest soil profile is composed, for the most part, of mature leaves from the trees in the stand. Through the process of decomposition the nutritional constituents of the litter are returned to the soil where, after decomposition, they enter into the

soil solution and the exchange complex and thus become available for re-assimilation. The maintenance of forest soil fertility is contingent to a large degree on the return of the constituents of the litter to the soil and subsequent litter decomposition. The chemical composition of the litter is one of the most important factors affecting the rate and type of decomposition.

The mature undecomposed leaves of nine species of forest trees, growing on Georgeville silt loam in the Duke Forest, were analyzed for total nitrogen, carbon, calcium, and ash content. The nine species were: loblolly pine, shortleaf pine, white oak, black oak, red gum, red cedar, red maple, yellow poplar, and dogwood.

White oak leaves had the highest total nitrogen content, 1.246 per cent, and red maple leaves contained the least nitrogen, 0.497 per cent. The nitrogen content of the other species decreased regularly in the following order: red cedar, black oak, dogwood, shortleaf pine, loblolly pine, yellow poplar, and red gum. The differences between nitrogen contents of all species were statistically significant as measured by three times the standard deviation of the difference.

Differences in carbon content were not great. Loblolly pine leaves contained the most carbon, 53.15 per cent, and dogwood leaves contained the least, 48.30 per cent.

The calcium content on the basis of oven-dry weight separates the nine species into two categories: (1) those with a relatively high calcium content, 2.0 to 3.5 per cent, which includes yellow poplar, red cedar, and dogwood in increasing orders; and (2) those with a relatively low calcium content, 0.4 to 1.1 per cent, including the other species with loblolly pine containing the least.

Leaves of dogwood and white oak contain rather large amounts of ash, 7.0 to 8.0 per cent, while the pines are low in ash, 3.0 to 3.5 per cent.

The Embryo Sac of Acorus Calamus L. MURRAY F. BUELL.

In the course of development of the orthotropous ovule of *Acorus Calamus L.* two integuments are produced. Of the two-layered nucellus, the outer layer develops into a perisperm; a remnant of the inner layer remains at the base, part of it forming a "postament." Directly from the archesporial cell is produced a tetrad of megaspores, the basal one of which develops into a normal eight-nucleate embryo sac. The two synergids disappear early, and the antipodals frequently increase to as many as five. By a division of the fusion nucleus the embryo sac is

divided into a small basal and a large micropylar cell, following which fertilization takes place. The endosperm is of the basal apparatus type. The cylindrical embryo finally lies in the axis of the seed.

Geometrical Transformations. EDWARD KASNER.

The paper discusses the geometry of "Turns" and "Slides." A turn T_α is defined as the operation of turning all elements (x, y, p) through a constant angle α. A slide S_a as sliding all elements, in the direction of the element, a distance K.

A discussion is given of the 3 parameter group $T_\alpha S_\kappa T_\beta$ called whirls. There exists a larger group, called the Turbine group, containing 15 parameters, possessing the property that a turbine is transformed into a turbine. It is pointed out that here we do not have point transformations but elements transformations, and that, in general a curve is not transformed into a curve but into a "series" of elements; a point also is transformed into a series (American Journal of Mathematics, 1916).

The work is then generalized to the theory of isogonal series and equitangential series. We are thus led to new groups related to conformal and equilong transformations.

Some Remarks on Stieltjes Integrals. F. G. DRESSEL.

The paper discusses the interrelations of the Young- and Radon-Stieltjes integrals. It concludes with the theorem

$$\int_0^1 l(y)df(y) = \int_0^1 l(y)Dfdg(y)$$

where Df is the derivative of the function of limited variation f(y) taken with respect to the monotone increasing function g(y), and l(y) is bounded and Borel measurable.

Some Theorems on Tensor Differential Invariants. J. LEVINE.

In this paper it is shown that in a relation of the form

$$T_{c\cdots d}^{a\cdots b}\left(\bar{g}_{ij;\cdots};\ \frac{\partial^m \bar{g}_{ij}}{\partial \bar{x}^{k_1}\cdots\partial \bar{x}^{k_m}}\right) u_a^p \cdots u_b^q = \phi(u) T_{r\cdots s}^{p\cdots q}\left(g_{ij\cdots};\ \frac{\partial^m g_{ij}}{\partial x^{k_1}\cdots\partial x^{k_m}}\right) \cdot u_c^r \cdots u_d^s$$

where g_{ij} defines the metric of a metric space and $u_j^i = \dfrac{\partial x^i}{\partial \bar{x}^j}$. The $\phi(u)$ is a power of the Jacobian of the coördinate transformation. The proof is based on the partial differential equations which tensor invariants satisfy, these equations being found in a paper by T. Y. Thomas and A. D. Michal, Annals of Mathematics (1927).

On the Equations of Conics of r-point Contact. J. W. LASLEY, JR.

The general equation of the conic together with the first five equations obtained by differentiation with respect to x give us a set of equations, the last three of which have as their resultant Monge's differential equations. If the fourth and fifth of these equations are solved for certain ratios, and the results placed in the first three, we obtain expressions for the coefficients of the general equation of the second degree as differential forms involving x,y,p,q,r, and s, where each of the foregoing is the derivative of the preceding as to x. If now we merely replace these quantities by correspondingly formed quantities x,Y,P,Q,R, and S for any point of a general curve, we obtain the equation of the conic of 5-point contact at that point. If we replace x, y, p, q, and r by x, Y, P, Q, and R, leaving s free, we obtain the equation of the pencil of penosculating, or 4-point conics. Continuing in this way we are able to obtain all conics of r-point contact for $r = 5$, or less.

Algebraic Systems. J. M. THOMAS.

The paper develops a new method for solving systems of simultaneous algebraic equations. It employs the resultant of two polynomials to give a reduction algorithm by means of which any algebraic system S of equations and inequations is factored in the form $S = T_1 T_2 \cdots T_l$, where the T's are *simple systems*, having special properties. In particular, (1) the fundamental theorem of algebra can be applied to each factor T; (2) the roots of each T are simple; and (3) there is an easy test for equivalence of systems when they are resolved into simple factors.

The factorization into simple factors is not unique. *Prime systems* are defined. The resolution into prime systems is essentially unique, but there is no algorithm for effecting the factorization, which in particular requires finding the irreducible factors of a polynomial.

The features of the present treatment which seem to be especially advantageous are (1) the systematic discussion of inequations along with equations; (2) the elimination of multiple roots; and (3) the applicability of the reduction algorithm without previous linear transformation of the indeterminates.

The Effects of Acids and Bases upon the Infra-red Absorption Bands of Water. E. K. PLYLER AND E. S. BARR.

Previous studies have shown that there are present absorption bands in solutions of acids and bases which are characteristic of the ions that are present. Also bands are produced by the undissociated molecules. In this study attention has been placed upon the change in intensity

and position of the water bands as produced by the presence of the acids and bases. It has been found that the intensity of the bands due to water is increased by the presence of the acids and bases. Also there is usually a shift in position of the water bands when measured in solutions.

Heat Transfer across an Interface Between Oil and Water. C. M. HECK AND G. W. BARTLETT.

The heat flow in each direction across an oil-water interface was found to be associated with a sudden difference in temperature at the interface amounting to about two degrees for the conditions in two designs of apparatus used. In one case the heat was applied at the bottom of a tall cylinder half filled with oil and half with water; temperature gradients were read throughout the whole length of both liquids and across the interface. In the other a short cylinder closed by a steam reservoir at the top and a cold water-circulating reservoir at the bottom had a set of opposed thermocouples, one as much above the interface as the other was below, rotated about an axis of support in the interface. The difference in temperatures of the two junctions were plotted against their distance apart as they approached the interface. Each quadrant of a complete revolution produces a curve extending from a projection onto the zero-drop axis to a maximum value. These curves are straight lines in pairs displaced apart about five degrees in temperature with the two in each pair practically coincident. In each liquid extrapolation of the temperature gradients back to the interface indicates in each of the above types of apparatus a discontinuity of temperature which discontinuity value is inversely related to the amount of disturbance at the interface by convection and agitation. The discontinuity indicated is negative when the direction of heat flow is from oil to water and positive when the flow is in the opposite direction.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

October 8, 1935, to May 12, 1936

362nd Meeting, October 8, 1935

W. C. Davison: *Medical Problems Peculiar to North Carolina.*

A number of diseases common in North Carolina were described. These included "blue gum negro bite," caused by spirochetes and curable by arsphenamine, fungus infections caused by blastomycetes or actinomycetes and curable in most cases by the administration of iodides, and lung abscesses following amebic dysentery. With regard to pellagra, it is now believed to be a resultant of three factors: (1) a dietary deficiency; (2) exposure to sunlight; (3) intestinal infections. North Carolina is second to Alabama in the incidence of pellagra and it is most prevalent in the month of June in this state.

The treatment of arthritis by artifical inducement of fever was described and the question was raised whether fever may not be nature's method of combatting a diseased condition.

The use of the ketogenic diet for the treatment of epilepsy was discussed and it was stated that this treatment causes improvement in 50–75 per cent of the cases.

The recent epidemic of poliomyelitis was discussed. This epidemic was unusual in that it reached its peak in early June although most previous epidemics reached their peak in September.

In conclusion some remarks were made on intestinal parasites, empyema, anemia, Malta fever, and infant mortality.

363rd Meeting, November 12, 1935

G. R. MacCarthy: *Magnetic Anomalies and Geologic Structures on the Carolina Coastal Plain.*

A magnetically disturbed zone, roughly parallel to the coast, has been outlined on both sides of the North-South Carolina border. This zone consists of a number of sub-parallel strips of alternately high and low intensity in the vertical component of the earth's magnetic field. It has been traced from the vicinity of Myrtle Beach to Wilmington,

and apparently continues further in a northeasterly direction. This disturbed zone probably represents a folded and perhaps a fractured belt in the crystalline basement rocks which are buried under about 2000 feet of Coastal Plain sediments in this region, and seems to be very similar to the disturbed zone which has been reported from the Florida peninsula.

The complete paper will appear in an early issue of the "Journal of Geology."

EDWARD MACK, JR.: *The Size and Shape of Molecules.*

Chemists and physicists have speculated about the existence of particles of matter for a very long time, and have used the idea of the atom and the molecule in their thinking and theorizing, even though there was no very definite evidence for the objective existence of such particles. Within the past fifty years, however, a great mass of rather convincing evidence has been accumulating, which leads to the view that atoms and molecules actually do exist. Indeed it is possible to deduce, by using certain physical methods, the sizes and the shapes of atoms and molecules.

The most powerful tool, so far developed, for determining the dimensions of these particles and something about their shapes is the method of x-ray analysis. (Here was given a brief description of the physical principle involved, and several scaled models were shown of the crystal lattices of sodium chloride, copper, iron, iodine, diamond, graphite and benzene.)

Another approach to size and shape, especially in the case of long hydrocarbon chain molecules with a polar, water-soluble, group at one end of the molecule, is the oil-film method developed in this country largely through the genius of Dr. Langmuir. (Here a blackboard description was given of the orientaton of such stick-like molecules on a water surface, and the method was presented for estimating the cross-section and the length of the molecules from the area and thickness of the oil-films.)

Of the half-dozen other methods which may be employed, one further method was described, namely the approach through measurement of the rate of diffusion of a volatile substance into air or the rate of viscous flow of a volatile substance down a capillary tube. From diffusion coefficients or from viscosity coefficients (at several temperatures) the collision areas of the molecules may be calculated. By matching these

areas against the average shadow areas of models, made to scale, definite conclusions about size and shape of the molecules may be reached.

364TH MEETING, DECEMBER 10, 1935

T. P. NOE, JR.: *The Determination of Stress in an Elastic Body.*

The principle of least work, developed by Castigliano, states that the internal work done in any structure by the application of outer forces will be the least possible, consistent with equilibrium. The application of this principle to the solution of statically indeterminate reactions and stresses in redundant members of trusses is quite familiar to engineers. However, the principle may also be used as a criterion for selecting from a number of possible cases the most probable distribution of stress intensity over a given cross section. The truth of the result obtained will depend upon whether the correct distribution has been included in the group tested.

As an illustration, the distribution of stress in a curved beam, subjected to pure bending, will be considered. For this case there are two variations of stress intensity that have been advanced and used by engineers: namely, that of linear variation (planar stress) and that of hyperbolic variation (planar strain). The two assumptions yield quite different results and only recently has it been determined by experiment that the hyperbolic variation is correct. The same result may be obtained by comparing the value of the total internal work computed on the basis of the two proposed distributions, the hyperbolic variation producing the least value for the internal work when the curved beam is subjected to a known external loading.

The fact that the internal work serves so well as a selective process is all the more interesting because the value of the internal work varies but slightly as the type of stress distribution considered is changed radically.

JOHN A. WHEELER: *The Formation of the Chemical Elements.*

Chemical analysis of samples of the earth's crust, of meteorites, and spectrochemical studies of the atmospheres of the sun and other stars give evidence that on the whole the earth is similar in its composition to the universe as a whole. Figures kindly supplied by Professor G. von Hevesy lead to the following estimates of the abundance of the more common isotopes in the solar system:

$^{16}_{8}$ O	52%	$^{29}_{14}$ Si	0.8%
$^{23}_{11}$ Na	0.6	$^{30}_{14}$ Si	0.8
$^{24}_{12}$ Mg	10	$^{32}_{16}$ S	2.6
$^{25}_{12}$ Mg	1.3	$^{40}_{20}$ Ca	0.9
$^{26}_{12}$ Mg	1.3	$^{54}_{26}$ Fe	0.6
$^{28}_{14}$ Si	12	$^{54}_{26}$ Fe	12

Heavy atoms, in comparison, are relatively rare; for example, only 0.0000026% of all atoms are estimated to belong to the species $^{209}_{83}$ Bi.

Experiments have shown in the past few years that fast charged particles (protons, $^{1}_{1}$H; deuterons, $^{2}_{1}$H; and alpha particles, $^{4}_{2}$He) are capable in close collisions of transmuting the lighter nuclei into heavier ones, often with the release of much energy. Objections to an explanation of element building on this basis are two in number: it has not proved possible in the laboratory to transmute the elements of medium and high atomic weight in this way; and at stellar temperatures, of the order of 10,000,000 degrees absolute, the charged particles are moving with an energy a hundred thousand times too small to be effective in collisions with light nuclei.

Promise of at least a partial accounting for the formation and relative abundance of the chemical elements comes from experiments of Enrico Fermi in 1934–35. Slow neutrons were found strikingly effective in transmutations of the type $^{1}_{0}n + {}^{127}_{53}I \rightarrow {}^{128}_{53}I$, where the heavy isotope formed is unstable and disintegrates, giving an element of atomic number one unit higher, $^{128}_{54}$ Xe, and an electron. So efficient are neutrons in producing nuclear changes that the presence of only a small proportion of them within a star would very quickly give rise to an extremely large liberation of heat, through the transmutations they bring about. Spectrochemistry does not tell whether neutrons are present in the stars. We may hope in the next few years to learn the answer to this question, and to trace in detail the successive changes through which the heaviest elements are built up from the lightest.

365TH MEETING, JANUARY 14, 1936

(Joint meeting with the Philological Club)

J. O. BAILEY (English): *Science—Preserver or Destroyer? Some Literary Futurities.*

366TH MEETING, FEBRUARY 11, 1936

R. W. BOST: *A Decade of Organic Chemistry.*

A review of some of the most important developments in organic chemistry during the last decade was given. The following topics were discussed: vitamins, hormones, anesthetics, disinfectants, perfumes, detergents, cracking of hydrocarbons, synthetic fuels, refrigerants, plant stimulants, synthetic resins, plant pigments and insecticides.

367TH MEETING, MARCH 3, 1936

E. W. MCCHESNEY: *The Analysis of Proteins.*

The proteins are perhaps the most important compounds found in living matter, and it is essential to the progress of biological chemistry that their structures and composition should be elucidated. In 1820 Braconnot discovered the first amino acid to be found in a protein; namely, glycine. The discovery of others followed rather rapidly until it became apparent about 1900 that proteins were made up entirely of alpha-amino acids. This led Fischer and Hofmeister to propose the "peptide" hypothesis of protein structure, which remains the most satisfactory conception. The recent work of Astbury on the X-ray diagrams of proteins tends to lend further support to the view.

Although accurate methods have been devised for the determination of some of the amino acids, our knowledge of the composition of many of the proteins is far from complete, ten to forty percent usually being unaccounted for. The remainder may represent undiscovered amino acids or analytical inaccuracies.

A fruitful method of study has been the separation of groups of amino acids having similar properties. A good example is the method of Foreman for the dicarboxylic amino acids. This aided Dakin in his discovery of hydroxyglutamic acid. In an attempt to repeat Dakin's work, the author found that casein yields a large fraction of amino acids which have water-insoluble barium salts. The principal component of this fraction is glutamic acid, but hydroxyglutamic acid is present and aspartic acid is absent. In addition a compound precipitable by phosphotungstic acid is present and it appears to be different from anything

previously described. It is not one of the diamino acids, and gives a strong diazo reaction. Further work with a view toward determining its nature is in progress.

DONALD P. COSTELLO: *Some Effects of Centrifugal Force on Marine Eggs.*

During the past 30 years the centrifuge has proven to be an instrument of extraordinary delicacy for bringing about a new distribution of the formed materials in certain living cells. The egg cells of many animals after being subjected to such treatment show a characteristic distribution of the fat, the yolk, and the pigment. The forced movement of these components through the egg, displacing the protoplasm, does not seem to injure the living substance, nor impair greatly its capacity for development. By the use of the centrifuge it has been possible to study many problems connected with the possible rôle of certain substances which might be thought to determine the future course of development.

The centrifuge used was an air-turbine ultracentrifuge of the Beams type, which gave forces up to 270,000 times gravity. Some unfertilized eggs of the marine worm, Nereis, centrifuged at 250,000 times gravity for 15 minutes, or at 100,000 times gravity for 60 minutes, subsequently developed, apparently normally.

The accumulation of vital dyes in particular formed cell components, rather than their coloration of the hyaline protoplasm, has also been demonstrated by the use of these high centrifugal forces. This indicates that the methods of measurement of the hydrogen ion concentration of hyaline protoplasm, employing such dyes, are completely inadequate.

Other problems related to the formation of the fertilization membrane have been studied in eggs of other marine forms using these high centrifugal forces.

368TH MEETING, APRIL 14, 1936

J. N. COUCH: *Some Recent Advances in Botany.*

369TH MEETING, MAY 12, 1936

H. M. BURLAGE: *The Development of the United States Pharmacopoeia and Formulary.*

Beginning with the Period of the Egyptian Papyri (previous to 1000 B. C.) works dealing with medicines and their preparations were discussed. From 1000 B. C. to 1000 A. D. was the period of works of individuals; 1000 A. D. to 1500, the period of Antidotariums; 1500 to 1800, City Pharmacopoeias; and 1800 to date, National Pharmacopoe-

ias. The development of the U. S. Pharmacopoeia was discussed through the present eleventh revision, with emphasis on the work of revision, the pharmacopoeial conventions and their compositions, important changes and trends and a comparison of these changes, trends and contents with other National Pharmacopoeias was discussed.

The National Formulary—previously called the National Formulary of Unofficial Preparations and a companion work of the U. S. Pharmacopoeia—is a product of the American Pharmaceutical Association. The ways and means of its development and revision were discussed. The relationship of these two national works in the enforcement of the Pure Foods and Drug Act was emphasized.

The following officers were elected for the year 1936–1937:

President—R. W. Bost.

Vice-President—English Bagby.

Secretary-Treasurer—E. L. Mackie.

The permanent secretary, E. T. Browne, and the editors of the Journal, W. C. Coker, H. V. Wilson, and Otto Stuhlman, continue in office.

ESTUARINE ANIMALS AT BEAUFORT, NORTH CAROLINA

By A. S. Pearse

Plates 15 and 16

INTRODUCTION

Beaufort is very favorably situated for the study of estuarine animals. It is near the open sea, but protected by Shackleford and Bogue Banks; it is surrounded by sounds, flats, marshes, and estuaries. Naturalists have long visited Beaufort. As early as 1860 Gill and Stimpson worked there; Coues and Yarrow were stationed at Fort Macon and Bogue Bank in 1871–1872 and published a list of animals that they found. In 1902 the present laboratory building of the United States Bureau of Fisheries was opened on Piver's Island. During the three preceding years the Bureau had maintained a laboratory in temporary quarters within the town of Beaufort. Since 1880, when the well known work of the marine laboratory of the Johns Hopkins University under Professor W. K. Brooks began, many scientific men have carried on investigations, and the flora, fauna, and hydrography are therefore well known.

The writer first worked at Beaufort during the summer of 1928. Grateful acknowledgment is made to Miss Sophie Dehler who helped with field and laboratory work at that time. In the spring and summer of 1934 Mr. Henry Hatsell gave excellent service as assistant and Miss Elizabeth Huntley helped during part of the summer. Dr. S. F. Hildebrand and Dr. H. F. Prytherch, Directors of the Beaufort Biological Station, United States Bureau of Fisheries, Captain Charles Hatsell and other members of the staff at the station did everything possible to help make the work successful. Many specialists have kindly identified specimens. For such gratuitious services acknowledgment is made as follows: plants, H. L. Blomquist, H. J. Oosting; foraminiferans, J. A. Cushman; trematodes, E. W. Price; nematodes, B. G. Chitwood; polychaetes, A. L. Treadwell; leeches, J. P. Moore; echinoderms, H. L. Clark; molluscs, Henry van der Schalie, P. Bartsch; copepods, C. B. Wilson; ostracods, Norma C. Furtos; barnacles, J. P. Visscher; amphipods, C. R. Shoemaker; isopods, J. O. Moloney; malacostracans, Mary

J. Rathbun, W. L. Schmitt; mites, Ruth Marshall; spiders, A. M. Chickering; insects, C. S. Brimley; collembolans, J. W. Folsom; odonates, P. P. Calvert, Jay R. Traver; bugs, H. B. Hungerford; orthopterans, A. N. Caudell; beetles, H. S. Barber, A. G. Bovig, L. L. Buchanan, W. S. Fisher, H. Morrison, C. F. W. Muesebeck, P. W. Oman; dipterans, H. G. Barber, C. T. Greene, G. A. Sandhouse, A. Stone. Mr. Richard Bellaire rendered good service in entering records.

In 1928 considerable time was spend in testing the ability of various animals to live when transferred to sea water or dilutions of it, but some collecting was done. In 1934 most of the time was devoted to collecting specimens and samples. In both years observations were made at twenty-two stations and in 1934 determinations of salinity, oxygen, hydrogen-ion concentration, and temperature were made continually.

STATIONS STUDIED

The following list gives the localities where observations were made and where collecting was done.

1. Shackleford Bank, beach on open sea.
2. Fort Macon; flats and small lagoon on Bogue Sound.
3. Shackleford Bank, beach on Back Sound.
4. Breakwater north of Fort Macon.
5. Mullet Pond, Shackleford Bank.

5A. Cattail Swamp, East of Mullet Pond.

6. Bogue Sound.
7. Bird Island Shoal.
8. Town Marsh.
9. Piver's Island.
10. Lenoxville Road, ditches and muddy shore.
11. Calico Creek.
12. John L. Godett's Sound.
13. North River.
14. Newport River.
15. Core Creek.
16. Eastman's Creek, off Core Creek.
17. Adam's Creek and Core Creek Canal.
18. Harlowe Creek.
19. Morton's Mill Pond.
20. Creek above Morton's Mill Pond.
21. Open Grounds.
22. Carteret Lodge Pond.

VARIATIONS IN PHYSICAL FACTORS

The stations just listed range from the open ocean to freshwater streams and ponds where salt-water never enters, and which lie twenty miles from the coast. When onshore winds prevail for several days salt water may back up into the waters of all the stations studied except the last three (20, 21, 22). On the other hand with prolonged offshore winds the waters of streams near the coast (11, 16, 18, 19) may remain quite fresh. Mullet Pond (5) is of interest because it lies on a narrow (0.5 mile) bank covered with moving sand dunes, and is close to the open sea. Its salinity varies greatly (0.04–26.94 0/00). When there are no high tides for a long period the little swamp (5A) and stream to the east feed nearly fresh water (S:0.30–0.84 0/00) into it; when high tides inundate the flats below it the water becomes quite salty. Temperatures of course vary with seasons and in severe winters ice forms in rather saline waters, but most marine and freshwater animals in the region studied escape freezing by burrowing into sandy or muddy bottoms. Hydrogen-ion concentrations also vary because of winds, floods, temperature, changes in salinity, depth, and other factors. The following observations show this:

Carteret Lodge Pond, June 28, 10:00 A.M.
Surface, S 0.05 ‰, T 31.1°C., pH 6.2.

Carteret Lodge Pond, June 28, 12:00 A.M.
Surface: S 0.05 ‰, T 32.5°C., pH 6.4, O_2 3.43.
Bottom, 2 ft.: S 0.05 ‰, T 29.0°C., pH 6.3, O_2 2.09.
Bottom, 6 ft.: S 0.05 ‰, T 26.9°C., pH 6.2, O_2 1.48.

Stream from Open Grounds to North River, July 3.
Mouth
Surface: S 20.10 ‰, T 30.4°C., pH 6.8, O_2 5.18.
Bottom: S 21.36 ‰, T 31.2°C., pH 6.8, O_2 5.19.
Middle
Surface: S 0.07 ‰, T 28.5°C., pH 7.4, O_2 3.02.
Bottom: S 0.07 ‰, T 27.3°C., pH 7.2, O_2 2.92.
Headwaters; depth, 4 in.
S 0.07 ‰, T 30.0°C.

Mullet Pond, July 11.
Surface: S 26.10 ‰, T 27.3°C., pH 8.6+.
Bottom: S 24.92 ‰, T 27.0°C., pH 8.6+.

Morton's Mill Pond, July 5.
Surface: 20.0 ‰, 33.8°C., pH 8.4, O_2 8.87.
Bottom: 20.8 ‰, 32.1°C., pH 8.2, O_2 6.39.

Oxygen was usually present in considerable quantities but the data just given show that it was at times somewhat lower near the bottoms of small ponds and streams than at the surface. Carter (1934) found similar conditions in British Guiana. The writer's figures represent cubic centimeters per liter.

Tides at Beaufort normally have a range of 2 ft. 7 in., but spring tides are about 3 ft. 6 in. and during great storms tides may reach 5 or 6 feet.

LIST OF ANIMALS OBSERVED

Many species in the following list are not in the card catalogue of the Beaufort fauna maintained at the Beaufort Biological Station, United States Bureau of Fisheries, and many there listed have not been seen by the writer. The two lists should therefore supplement one another. When the card catalogue contains information as to general distribution of a species studied by the writer at Beaufort, it is appended to species mentioned in this list.

Protozoa

Foraminiferida

Elphidium poeyanum (d'Orbigny)

Mullet Pond, in tow net; July 11, 1935.

Rotalia beccarii (L.) *parkinsonia* (d'Orbigny)

Morton's Mill Pond (S:8.95), May 1. Mullet Pond, June 26, (S:3.60), July 11 (S:3.20, 0.3); Carteret Lodge Pond (S:0.05), June 28, 1934.

Porifera

George and Wilson (1921) describe 17 species of sponges from Beaufort Harbor and vicinity. None of these was identified during the present study.

Coelenterata

Hydrozoea

Cordylophora lacustris Allman

Open Grounds; ditch (S:8.73), June 28, 1928.

Eudendrium carneum Clarke

Core Creek on Libinia carapace; June 16, 1928.

Hydractinia echinata Fleming

Piver's Island; June 13, 1928; on hermit crab's shell.

Physalia pelagica (L.)

Beaufort Inlet; July 23, 1928.

Tubularia crocea Agassiz

Piver's Island; August 13, 1928.

Nemopsis bachei Agassiz

Morton's Mill Pond (S:8.95); May 1, 1934. Several in tow, May, 1914.

Scyphozoea

Dactylometra quinquecirrha (Desor)

Morton's Mill Pond (S:20.4); July 5, 1934.

Anthozoea

Gorgonia verrucosa Agassiz

Piver's Island; August 10, 1928; Bogue Sound, March 30, 1934.

Edwardsia sp.

Young anemones of this genus were found, Mullet Pond, April 30 (S:9.11) and June 18 (S:12.54); Town Marsh, June 27.

Sagartia luciae Verrill

Core Creek Canal, June 14, 1928; Breakwater north of Fort Macon, June 25, 1928.

Astrangia danae Agassiz

Piver's Island, June 13, 1928; Bogue Sound, March 30, 1934.

Ammophilactis rapiformis Verrill

Sheep's Head Shoal; June 14, 1928. Cast up on Fort Macon beach after storm.

Ctenophora

Mnemiopsis leidyi Agassiz

Sheep's Head Shoal; June 15, 1928. Piver's Island, March 31; Morton's Mill Pond, June 19 (S:8.95), May 1 (S:5.0); Harlowe Creek, June 22 (S:3.25); Mullet Pond (S:3.70); June 26, 1934. Often common.

Platyhelmintha

Turbellarea

Bdelloura candida (Girard)

On *Limulus polyphemus* (L.) Breakwater Flat, June 13, 1928; Bogue Sound, March 31; Town Marsh, June 27, 1934.

Trematodea

Levinseniella jagerskioldi Travassos

Piver's Island; April 1, 1934, in *Uca pugilator* (Bosc).

Nemertea

Cerebratulus lacteus Leidy

Core Creek; June 14, 1928. Under stones on Shackleford Bank.

Nemata

Acuarine larvae

In visceral cysts in *Uca minax* (Le Conte), Harlowe Creek, April 3, 1934.

Metaparoncholaimus sp.

Mullet Pond, July 11, 1934 (S:19.8).

Spilophorella sp.

Mullet Pond, July 11, 1934 (S:19.8).

Bryozoa

Bugula turrita (Desor)

Piver's Island, June 12, 1928.

Schizoporella unicornis (Johnston)

Piver's Island; June 12, 1928.

Echinodermata

Asterea

Asterias forbesi (Desor)

Town Marsh; June 13, 1928.

Ophiurea

Ophiothrix angulata Say

Piles on bridge; June 12, 1928. Common.

Ophioderma brevispina (Say)

Bird Island Shoal, August 13, 1928; Bogue Sound, March 30, 1934. Common.

Echinoidea

Arbacia punctulata (Lamarck)

Piver's Island, June 12, 1928. Common.

Mellita quinquiesperforata (Leske)

Breakwater Flat, June 13, 1928; Bogue Sound, March 30; Bird Island Shoal, June 25, 1934. Abundant on suitable bottoms along open sea.

Holothurea

Thyone briareus (Lesueur)

Sheep's Head Shoal, June 15, 1928. Rather common.

Leptosynapta inhaerens (O. F. Müller)

Breakwater Flat; June 13, 1928. Not very common; larvae at times abundant in towings.

Annelida

Polychaetea

Autolytus varians Verrill

Core Creek Inlet; June 16, 1928.

Nereis limbata Ehlers

Breakwater Flat, June 13, 1928; Morton's Mill Pond, April 3 (S: 2.48), June 19 (S:5.6); Calico Creek, May 2 (S:16.5); July 6 (S:32.4); Core Creek Canal, July 10 (S:3.55), 1934.

Leptonereis culveri Treadwell

Mullet Pond, June 18 (S:13.0), July 11 (S:3.2, 19.8), 1934.

Nephthys bucera Ehlers

Piver's Island, June 12, 1928.

Onuphis magna Andrews

Bogue Sound, March 30, 1934 (S:27.77). Common on Bird Island and Shark Shoals.

Onuphis cuprea (Bosc)

Breakwater Flat; June 13, 1928. Piver's Island.

Glycera americana Leidy

Piver's Island; June 13, 1928; Mullet Pond, June 26, 1934 (S:12.6).

Chaetopterus pergamentaceus Cuvier

Breakwater Flat, June 13, 1928. Common in appropriate places.

Amphitrite ornata (Leidy)

Core Creek, June 14, 1928. Common.

Hydroides hexagonus Bosc

Piver's island; June 12, 1928. Common on old shells.

Hirudinea

Placobdella rugosa (Verrill)

Carteret Lodge Pond, June 28, 1934; S:0.05.

Myxobdella lugubris Leidy

Mullet Pond, April 1, June 26, July 11, 1934; common on *Callinectes sapidus* Rathbun.

Piscicolaria, juv.

Echiurea

Thalassema mellita Conn

Breakwater Flat; June 13, 1928.

Mollusca

During 1933 Dr. Henry van der Schalie, Museum of Zoology, University of Michigan, worked at the Beaufort Biological Station. He has furnished a list of molluscs identified by him and Dr. W. J. Clench. These are preceded by a star(*) in the list. Other specimens were referred to Dr. van der Schalie for identification. Jacot (1921) published a list of the marine molluscs of Beaufort and reviewed the work of previous writers.

Gastropodea

**Diadora alternata alternata* Say

Breakwater Flat, June 13, 1928. Common on trestle near Morehead City.

**Turbo castaneus castaneus* Gmelin

Town Marsh, July 18, 1928.

**Melanella conoidea* Kurtz & Stimpson

**Littoridina monroensis* (Frld.)

Calico Creek, May 1 (S:8.95), June 21 (S:29.2); Morton's Mill Pond, June 19 (S:4.9); Mullet Pond, June 18, (S:12.54), June 26 (S:12.6); July 11, 1934 (S:19.8).

**Pyramidella crenulata* Holmes

**Natica pusilla* Say

**Sinum perspectivum* Say

Bird Island Shoal, August 10, 1928. Common on Bird Island Shoal.

**Polynices duplicata* Say

Piver's Island, June 12, 1928. Common in Beaufort Harbor.

**Calyptraea centralis* Conrad

**Crepidula fornicata* (Verrill)

Piver's Island, June 12, 1928; Mullet Pond, June 18, 1934. Common on Limulus and shells.

**Crepidula plana* Say

Piver's Island, June 12, 1928; Bogue Sound on Leniulus, March 31, 1934. Common on Limulus, shells of hermit crabs, etc.

Paludestrina tenuipes Couper

Above Morton's Mill Pond, May 1 (S:0.73); Calico Creek, May 2, 1934 (S:0.76).

**Littorina irrorata* Say

Piver's Island, June 12, 1928; Calico Creek, May 12 (S:16.52), June 21 (S:4.69), July 6 (34.1); John Godett Bay, June 19 (S:13.85); Core Creek Canal, July 10 (S:0.7, pH 6.8); July 12, 1934 (S:15.52). Common on sedges.

**Epitonium liumphreysii* Keiner

On drift from Shark Shoal.

**Triphora perversa nigrocincta* C. B. Adams

**Seila adamsii* H. C. Lea

**Strombus pugilis pugilis* L.

At times taken in seines outside the banks.

**Simnia uniplicata* Sowerby.

On piles and gorgonian corals.

**Cassis tuberosa* (L.)

**Cassis inflata inflata* Shaw

**Tonna galea* L.

**Ficus papyratia* Say

**Eupleura caudata caudata* Say

Piver's Island, June 12, 1928.

**Urosalpinx cinereus* (Say)

Piver's Island; June 12, 1928. Common on piles and breakwater at Shackleford Bank and Fort Macon.

**Thais floridana floridàna* Conrad

On Morehead trestle and Shackleford Bank.

**Anachis avara avara* (Say)

**Anachis obesa obesa* C. B. Adams

Common in Bogue Sound.

**Mitrella lunata lunata* Say

**Nassarius vibex* (Say)

Piver's Island, June 12, 1928. Common on Shark Shoal.

**Nassarius obsoleta* (Say)

Piver's Island, June 12, 1928. Abundant on mud flats generally.

**Nassarius trivitata* (Say)

**Busycon carica carica* Gmelin

Abundant on Bird Island and Shark Shoals.

**Busycon perversum perversum* (L.)

Reported by van der Schalie and Clench. Common on Bird Island and Shark Shoals and near Fort Macon Beach.

**Fasciolaria distans* Lamarck

Piver's Island, June 12, 1928. Common on mud flats near Piver's Island, Shark Shoal, etc.

**Marginella apicina apicina* Menke

**Oliva sayana sayana* Ravenel

Piver's Island, June 12; Town Marsh, June 13, 1928. On Shark and Bird Island Shoals; on sandy beaches.

**Olivella mutica* Say

Breakwater Flat, June 13, 1928. On Shark and Bird Island Shoals.

**Terebra dislocata dislocata* Say

Breakwater Flat, June 13, 1928. Common on shoals.

**Mangilia cerina* Kurtz & Stimpson

**Mangilia plicosa* C. B. Adams

**Acteocina canaliculata* Say

Elysia chlorotica Gould

Morton's Mill Pond, April 3, 1934.

Polygyra thyroides (Say)

Harlowe Creek, April 4, 1934.

Philomycus carolinensis (Bosc)

Open Grounds, ditch, July 2, 1934.

Succinea campestris Say

Open Grounds, ditch, July 2, 1934.

**Melampus lineatus* Say

Mullet Pond, June 22; North River, June 29, 1928; Harlowe Creek, April 2, June 22; Calico Creek, April 2; Open Grounds, July 2; Eastman's Creek, July 12, 1934.

Lymnaea columella Say

Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28. 1934 (S:0.05).

Physa pomilia hendersoni Clench

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Gyraulus dilatus Gould

Open Grounds, ditch, June 20, 1934 (S:0.3).

Ferrissia sp.

Open Grounds, ditch June 20, 1934 (S:0.3).

Pelecypodea

**Solemya velum* Say

Piver's Island, June 12, 1928. Common on shoals.

**Nucula proxima proxima* Sowerby

Nuculana acuta (Conrad)

**Arca campechiensis* Dillwyn

**Arca incongrua* Say

**Arca occidentalis* Philippi

**Arca secticostata* Reeve

**Arca transversa* Say

Abundant half a foot below low-water mark on muddy bottoms among eel grass; Horse Island. This is the commonest representative of the genus.

**Noetia ponderosa* Say

Breakwater Flat, June 13, 1928. On muddy bottoms; less common than last species.

**Eulima conoidea* Kurtz & Stimpson

In mud among dead shells; fairly common.

**Glycimeris pectinata* Gmelin

**Panopea floridana* Heilprin

**Atrina rigida* (Dillwyn)

Piver's Island, June 12, 1928. On muddy bottoms. Rather common.

**Atrina serrata* Sowerby

**Pteria eximia* Reeve

On gorgonian corals.

**Ostrea virginica* Gmelin

Newport River, June 29, 1928 (S:35.95); Adams Creek, April 2 (S:0.0); Harlowe Creek, June 22 (S:0.5), July 5 (S:20.0). Eastman's Creek, July 12, 1934 (S:15.5). Alongshore, largely found from a foot below tide mark upward.

**Ostrea equestris* Say

Found mostly from low-tide mark to depth of about ten feet.

**Plicatula gibbosa* Lamarck

**Pecten irradians irradians* Lamarck

Piver's Island, June 12, 1928. Bogue Sound, June, 1934. This is the common scallop among eel grass.

**Pecten gibbus gibbus* L.

By some thought to be synonymous with the last species.

**Anomia simplex* d'Orbigny

Breakwater Flat, June 13, 1928. Common on old shells.

**Mytilus exustus* L.

Breakwater Flat, June 13, 1928; Morton's Mill Pond, April 3 (S:12.48), May 1 (S:0.73), June 19 (S:3.79); Calico Creek, May 2 (S:21.4); Harlowe Creek, June 22 (S:2.08).

**Modiolus demissus demissus* Dillwyn

Piver's Island, June 12; Mullet Pond, June 22, 1928; Calico Creek, July 7 (S:33.5); Core Creek Canal, July 10 (S:18.9); Eastman's Creek, July 12 (S:15.5). This is the common mussel about Beaufort.

**Modiolus tulipus* Lamarck

**Lithophaga bisulcata* d'Orbigny

Bogue Sound, March 30, 1934. The boring "date shell" penetrates the shells of other molluscs.

**Modiolaria lateralis* Say

**Congeria leucophaeta* Conrad

**Polymesoda caroliniana* Bosc

Harlowe Creek, April 2 (S:0.0) June 22 (S:3.5); stream above Morton's Mill Pond, May 1 (S:0.73), July 9 (S:18.23); Core Creek Canal, July 10 (S:3.55). This clam lives in mud among the roots of water plants in swampy places along streams.

**Gouldia mactracea* Linsley

**Venericardia tridentata* Say

**Chama congregata* Conrad

**Echinochama arcinella* L.

**Divaricella quadriculcata* d'Orbigny

**Loripinus chrystomata* Philippi

**Cardium isocarida* L.

**Cardium muricatum* L.

Breakwater Flat, July 13, 1928. Fairly common along edges of channel between Beaufort and Bird Island Shoal.

**Trigonocardia serrata* (L.)

**Laevicardium mortoni* Conrad

**Dosinia discus* Reeve

Bird Island Shoal, July 16, 1934. Common about one foot below surface of bottom; Shark Shoal, etc.

Macrocallista nimbosa Solander

Breakwater Flat, June 13, 1928.

Musculium partumieum Say

Open Gounds, ditch, June 20 (S:0.5); Calico Creek, June 21 (S:0.17); Carteret Lodge Pond, June 28 (S:0.05).

**Macrocallista maculata* L.

**Pitar morrhuana* Linsley

**Chione cancellata* L.

Piver's Island, June 12, 1928.

**Chione latilirata* Conrad

The "dog clam" is common among eel grass.

**Venus mercenaria mercenaria* L.

Piver's Island, June 12, 1928. This is the common "hardshell" clam or "quahog" about Beaufort.

**Venus campechiensis campechiensis* Gmelin

Piver's Island, June 12, 1928.

**Venus ziczac* L.

Gemma gemma purpurea H. C. Lea

**Ptericola pholadiformis pholadiformis* Lamarck

Core Creek, June 25, 1934.

**Ptericola pholadiformis lata* Dall

**Tellina lintea* Conrad

**Tellina alternata* Say

On northwest end of Shark Shoal, just below low-tide mark.

**Tellina tenera* Say

Morton's Mill Pond, April 3 (S:2.48); Mullet Pond, April 30 (S:8.42); June 18 (S:12.54), June 26 (S:12.6); Core Creek Canal, July 10 (S: 18.9).

**Tellina versicolor* Cozzens

**Donax fossor fossor* Say

**Donax variabilis* Say

Abundant along the beaches of Bogue Sound.

**Tagelus gibbus* Spengler

On mud flats; common. This is the "short razor" clam.

**Tagelus divisus* Spengler

A small species abundant below low-tide mark on shoals near sea.

**Ensis directus* Conrad

Breakwater Flat; June 13, 1928. This is the "razor" clam.

**Mactra fragilis* Gmelin

**Spissula solidissima solidissima* Dillwyn

Abundant off Bird Island and Shackleford Shoals.

**Spissula solidissima similis* Say

Bird Island Shoal, July 16, 1928.

**Mulinia lateralis lateralis* Say

**Rangia canaliculata* Say

Sheeps' Head Shoal, June 15, 1928.

**Mya arenaria* L.

Found in turtle ponds on Piver's Island.

**Corbula contracta* Say

**Pholas costata* L.

Sheeps' Head Shoal, June 15, 1928.

**Martesia cuneiformis* Say

Bores in oysters and other molluscs.

Bankia gouldi (Bartsch)

Piver's Island, August 6, 1928; Bird Island Shoal, August 10, 1928: Core Creek, June 25, 1934.

Cephalopodea

Loligo pealei pealei Lesueur

Core Creek Inlet, June 16, 1928; June 25, 1934. This squid is often taken in trawls. Egg masses were common on Fort Macon beach, May 15, 1920.

Arthropoda

Crustacea

Hay and Shore (1918) published an account of the decapod crustaceans of the Beaufort region. Their list includes 153 species, many of which were collected in deep water offshore.

Crustacea

Cirripedia

Beaufort barnacles are well described in Pilsbry's (1916) monograph.

Balanus eburneus Gould

This acorn barnacle occurs chiefly below low-water mark; "often in brackish and even fresh water." During the present investigations it was often found far inland: Adam's Creek, April 2 (S:0.00); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:4.9); Harlowe Creek, June 22 (S:2.08); July 2 (S:26.0); Open Ground, July 3 (S:0.07); Eastman's Creek, July 12, 1934 (S:15.52).

Balanus improvisus Darwin

Usually below low-water mark. Found in a ditch in the Open Grounds, June 18, 1928 (S:0.07) with such freshwater animals as bream, largemouth bass, water bugs, gyrinids, and crayfishes.

Balanus amphitrite Darwin

Fort Macon Beach, June 27, 1928 (S:34.87).

Chelonibia caretta (Spengler)

From carapace of green turtle, Town Marsh, June 25, 1928; loggerhead turtle, Bogue Sound, May 11, 1936.

Dichelaspis mülleri Coker

In branchial cavity of *Uca minax* (Le Conte), Harlowe Creek, April 3, 1934 (S:0.01).

Cladocerida

Acantholeberis curvirostris (O. F. Müller)

Open Grounds, June 20, 1934 (S:0.4).

Alona affinis (Leydig)

Open Grounds, June 20 (S:0.4); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Simocephalus serrulatus Koch

Open Grounds, June 20 (S:0.4); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Copepodia

All copepods were identified by Dr. C. B. Wilson and are described in his paper (1932) on the copepods of the Woods Hole region.

Pseudocalanus elongatus Boeck

Stream above Morton's Mill Pond, May 1 (S:0.73); Calico Creek, June 21, 1934 (S:29.2).

Macrocyclops albidus (Jurine)

Calico Creek, May 2 (S:0.76); Morton's Mill Pond, June 19 (S:4.9); Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Macrocyclops fuscus (Jurine)

Carteret Lodge Pond, June 28 (S:0.05); Mullet Pond, July 11, 1934 (S:3.2).

Eucyclops serrulatus (Fischer)

Harlowe Creek, July 5 (S:26.0); Mullet Pond, July 11, 1934 (S:3.2).

Acartia clausii Giesbrecht

Mullet Pond, April 30 (S:8.42); Harlowe Creek, July 5 (S:26.0); Core Creek, July 12, 1934 (S:31.42).

Acartia longiremis (Lilljeborg)

Mullet Pond, April 30, 1934 (S:8.42).

Amphiascus pallidus Sars

Mullet Pond, June 18, 1934 (S:13.0).

Echinostoma normani T. & A. Scott

Mullet Pond, July 11, 1934 (S:19.8).

Ostracodia

All ostracods were identified by Dr. Norma C. Furtos.

Cypridopsis vidua vidua (O. F. Müller)

Morton's Mill Pond, May 1 (S:0.73); Calico Creek, May 1 (S:0.76); Carteret Lodge Pond, June 28 1934 (S:0.05).

Cyprinotus putei Furtos

Mullet Pond, July 11 1934 (S:3.2).

Physocypria globula Furtos

Stream above Morton's Mill Pond, May 1 (S:0.73); Calico Creek, May 1, 1934 (S:0.76).

Cypretta intonsa Furtos

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Candona annae Mehes

Open Grounds, ditch, June 20, 1934 (S:0.3).

Cytheridae, sp.?

Stream above Morton's Mill Pond, May 1 (S:0.73); Mullet Pond, June 26 (S:12.6), July 11 (S:3.2), stream below Morton's Mill Pond, July 9 (S:18.23).

Amphipodida

Mr. C. R. Shoemaker (*) identified many of the amphipods.

Orchestia platensis Kroyer

Piver's Island, June 12, 1928.

Orchestia grillus (Bosc)

Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19 (S:5.3); Mullet Pond, April 30 (S:8.42); stream above Morton's Mill Pond, July 9 (S:21.83); Core Creek Canal, July 10, 1934 (S:18.9).

Gammarus locusta (L.)

Stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

**Gammarus* sp. juv.

Calico Creek, June 21, 1934 (S:29.2).

**Eucrangonyx* sp.

Stream above Morton's Mill Pond, May 1, (S:0.73); Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Carinogammarus mucronatus* (Say)

Harlowe Creek, April 2 (S:2.16); Morton's Mill Pond, April 3 (S: 2.48), June 19 (S:5.3), Open Grounds, March 28 (S:0.00), Mullet Pond, April 30 (S:8.42), June 18 (S:0.00), June 26 (S:0.4), July 11 (S:19.8); John Godett Bay, June 19 (S:13.65).

Grubia compta (S. I. Smith)

Bogue Sound, March 30, 1934 (S:27.77).

Corophium cylindricum (Say)

Morton's Mill Pond, April 3, 1934 (S:2.48).

Caprella linearis L.

Bogue Sound, March 30, 1934 (S:27.77).

Isopodida

Mr. J. O. Moloney (*) identified certain species of isopods.

**Tanais* n. sp.

Mullet Pond, July 11, 1934 (S:19.8).

Leptochelia rapax Harger

Common in tubes in the mud in Mullet Pond, April 30 (S:8.42); June 18 (S:13.2); June 26 (S:12.6); July 11, 1934 (S:19.8).

Leptochelia savignyi Kroyer

Morton's Mill Pond, on submerged logs, April 3, 1934 (S:2.48).

Cyathura carinata Kroyer

Open Grounds, June 22, 1928; July 3 (S:0.07).

Cassidinidea ovalis (Say)

Morton's Mill Pond, April 3, 1934 (S:2.48).

Sphaeroma quadridentatum Say

Shackleford Bank, under stones, April 1, 1934.

Erichsonella attenuata (Harger)

Mullet Pond, among Enteromorpha filaments, April 30 (S:8.42), June 18 (S:12.8), July 11 (S:19.8), Morton's Mill Pond, June 19, 1934 (S:5.3).

Probopyrus pandalicola (Packard)

In branchial cavity of *Palaemonetes carolinus* Stimpson; Morton's Mill Pond, May 1 (S:8.95), June 19 (S:5.3), July 6 (S:18.23); Calico Creek, June 21 (S:0.16); Harlowe Creek, June 22, 1934 (S:3.5).

Leidya distorta (Leidy)

In branchial chamber of *Uca pugilator*, a male and a female together, Piver's Island, May 31, 1934.

Pseudione upogebiae Hay

In branchial cavity of *Upogebia affinis* (Say); Core Creek, June 14, 1928.

Asellus communis Say

Open Grounds, March 28, 1934 (S:0.0).

Decapodida

Quotations are from Hay and Shore (1918).

Natantina

Penaeus brasiliensis Latreille

Abundant in brackish creeks. Morton's Mill Pond, June 19 (S:5.3); Calico Creek, June 21 (S:0.16), July 6 (S:32.4); Core Creek Canal, July 10 (S:3.55); Harlowe Creek, June 22, 1934 (S:3.5).

Penaeus setiferus (L.)

Common in summer. Harlowe Creek, July 5, 26.0; Core Creek Canal, July 10, 1934 (S:3.55).

Palaemonetes carolinus Stimpson

"Abundant among eel grass and about the margins of marshes," Harlowe Creek, April 2 (S:2.16), June 22 (S:3.5); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19 (S:5.3), above Morton's Mill Pond, May 1 (S:0.73), July 9 (S:18.23), with eggs; Mullet Pond, April 30 (S:9.11), June 18 (S:12.54) June 26 (S:12.5), July 11 (S:0.3); Calico Creek, May 2 (S:0.76–21.4), June 21 (S:0.16); Core Creek Canal, July 10 (S:3.55); Eastman's Creek, July 12, 1934 (S: 15.52).

Palaemonetes vulgaris (Say)

Often associates with the last species. Calico Creek, July 6, 1934 (S:32.0).

Palaemonetes exilipes Stimpson

Mullet Pond, June 18, young (S:1.69); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Reptantina

Cambarus blandingii (Harlan)

Open Grounds, ditch, June 18, 1928 (S:0.3); July 3 (S:0.07).

Upogebia affinis (Say)

Core Creek Canal, June 14, 1928.

Emerita talpoidea (Say)

Sheep's Head Shoal, Shackleford Bank, June, 1928, 1934. Common on sandy beaches in certain localities.

Pagurus pollicaris Say

Bogue Sound, March 30, 1934, with eggs (S:27.77).

Hepatus epheliticus (L.)

Beaufort Breakwater, June 20, 1928.

Ovalipes ocellatus ocellatus (Herbst)

Sheep's Head Shoal, June 15, 1928.

Callinectes sapidus Rathbun

Open Grounds, ditch, June 22, 1928, July 3, 1934 (S:0.07); Bogue Sound, March 30 (S:27.77); Mullet Pond, April 1 (S:8.3), June 18 (S:0.05), June 26 (S:12.6), July 11 (S:32); Harlowe Creek, April 2 (S:2.16); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:5.3), July 5 (S:20.4); Creek above Morton's Mill Pond, July 9 (S:21.8); Calico Creek, June 21 (S:0.16); July 6 (S:34.1); Lenoxville Road, ditch, June 30 (S:28.6); Core Creek Canal, July 10 (S:0.7–18.9); Eastman's Creek, July 12, 1934 (S:15.5).

Rithropanopeus harrisii (Gould)

Open Grounds, ditch, June 28, 1928 (S:0.05); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:4.02); Harlowe Creek, June 22, 1934 (S:3.5).

Eurypanopeus depressus (Smith)

Beaufort Breakwater, June 25, 1928.

Neopanope texana sayi (Smith)

Piver's Island, June 24, 1928.

Panopeus sayi Smith

Core Creek, June 14; Piver's Island, pier, June 16, 1928.

Menippe mercenaria De Haan

Town Marsh, June 18, 1928; Bogue Sound, March 30, 1934 (S:27.77).

Pinnotheres ostreum Say

Piver's Island, June 15, 1928; in oysters.

Pinnotheres maculatus Say

Bogue Sound, March 30, 1934 (S:27.77).

Dissodactylus mellitae (Rathbun)

On sand dollars, Mellita; Bird Island Shoal; June 25, 1934.

Pinnixa sayana Stimpson

Bird Island Shoal, August 15, 1928.

Sesarma cinerea Say

Calico Creek, May 2 (S:16.52); Harlowe Creek, June 22 (S:3.3–0.5); Mullet Pond, July 11 (S:0.3); Core Creek Canal, July 10, 1934 (S: 18.9).

Ocypode albicans Bosc

Common on sandy beaches above high-tide mark, where it lives in burrows.

Uca minax (Le Conte)

Open Grounds, June 18; Sheep's Head Shoal, June 15, 1928; Harlowe Creek, April 2 (S:2.2–9.3), June 22 (0.5–3.3), July 5 (S:26.0); Morton's Mill Pond, April 3 (S:2.48); Calico Creek, May 2 (S:18.5), June 21 (S:0.16), July 6 (S:33.2); above Morton's Mill Pond, July 9 (S:12.64); Lenoxville Road, June 30 (S:28.44); Core Creek Canal, July 10 (S:0.07); Eastman's Creek, July 12, 1934 (S:15.52).

Uca pugnax (Smith)

Fort Monroe, marshes, June 27, 1928.

Uca pugilator (Bosc)

Core Creek Canal, June 14, 1928; Piver's Island, March 31; Mullet Pond, April 30 (S:8.42), July 11, 1934 (S:19.8).

Insectea

Collembolida

Identified by J. W. Folsom (*)

**Anurida maritima* Guérin

Shackleford Bank, under stones, June 16, 1934.

**Bourletiella spinata* Mac. G.

On water along stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

**Isotomurus palustris* Müller

On water along stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

Ephemerida

Identified by Dr. Jay R. Traver (*)

**Callibaetis* sp.

Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Caenis diminuta* Walker

Open Grounds, ditch, June 20 (S:0.03); Carteret Lodge Pond, June 28, 1934, (S:0.05).

Odonatida

Identified by P. P. Calvert (*)

**Enallagma* sp., juv.

Mullet Pond, June 18 (S:0.05), June 26 (S:12.6), July 11 (S:3.2, 19.8); Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Ischnura posita* (Hagen)

Open Grounds, ditch, June 20 (S:0.5), July 2 (S:0.05); stream above Morton's Mill Pond, May 1 (S:0.73), July 9, 1934 (S:15.5).

**Ischnura ramburii* Selys

Mullet Pond, April 30 (S:9.11), April 28 (S:8.42), June 18, June 26 (S:0.4), July 11 (S:0.3); Morton's Mill Pond, June 19 (S:5.3), July 9 (S:15.5); Open Grounds, ditch, June 20 (S:0.3, 0.5); Calico Creek, June 21 (S:0.16); Core Creek Canal, July 11, 1934.

**Ischnura verticalis* (Hagen)

Open Grounds, June 20, 1934 (S:0.3).

**Agrion maculatum* Beauvois

Open Grounds, stream, June 21, 1928, July 3, 1934.

**Pachydiplax longipennis* Burmeister

Mullet Pond, June 22, 1928; Open Grounds, ditch, June 20; Core Creek Canal, July 10, 1934.

**Anomalagrion hastatum* (Say)

Open Grounds, June 20; Calico Creek, June 21; Mullet Pond, June 28; stream above Morton's Mill Pond, July 9, 1934.

**Argia fumipennis* (Burmeister)

Open Grounds, ditch, June 20, July 3, 1934.

**Calopteryx maculata* (Beauvois)

Open Grounds, stream, July 3, 1934.

**Anthax sinuosa* Wied.

Morton's Mill Pond, June 19, 1934.

**Erythrodiplax berenice berenice* (Drury)

Mullet Pond, June 18, June 26, July 11; Morton's Mill Pond, June 19; Lenoxville Road, June 30; Open Grounds, ditch, July 2, July 3, 1934.

**Somatochlora linearis* (Hagen)

Eastman's Creek, July 12, 1934.

**Libellula auripennis* Burmeister

Morton's Mill Pond, June 19; Calico Creek, June 21; Carteret Lodge Pond, June 28; stream above Morton's Mill Pond, July 9, 1934.

**Libellula vibrans* Fabricius

Open Grounds, stream; July 3, 1934.

**Libellula incesta* Hagen

Carteret Lodge Pond, June 28, 1934.

**Libellula semifasciata* Burmeister

Open Grounds, June 20; Carteret Lodge Pond, June 28, 1934.

**Celithemis eponina* Drury

Carteret Lodge Pond, June 28, 1934.

**Erythemis simplicicollis* (Say)

Mullet Pond, April 30, June 18; Carteret Lodge Pond, June 28, 1934.

**Pachydiplax longipennis* Burmeister

Mullet Pond, April 30, June 26; Open Grounds, June 20; Carteret Lodge Pond, June 28; Core Creek Canal, July 10, 1934.

**Plathemis lydia* (Drury)

Calico Creek, June 21, 1934.

**Anax junius* (Drury)

Mullet Pond, July 11, 1934, nymph (S:0.3).

**Gomphaeschna furcillata* (Say) *antilope* Hagen

Open Grounds, June 20; stream above Morton's Mill Pond, July 9, 1934.

**Coryphaeschna ingens* (Rambur)

Heteropterida

Bugs were identified by H. B. Hungerford (*) and C. S. Brimley (**).

**Saldula reperta* Uhlman

Mullet Pond, July 11, 1934.

***Gerris nebularis* Drake & Hottes

Open Grounds, June 28, 1928.

**Gerris* sp., juv.

Stream above Morton's Mill Pond, May 1; Open Grounds, June 20, July 3; Carteret Lodge Pond, June 28, 1934.

***Arctocorixa* sp.

Mullet Pond, June 22, June 28, 1928, April 30, 1934.

**Hydrometra martini* Kirk

Open Grounds, ditch, June 20; Carteret Lodge Pond, June 28, 1934.

**Rheumatobates tenuipes* Meinert

Stream above Morton's Mill Pond, July 9, 1934.

Benacus griseus Say

Open Grounds, June 18; 1928**; Carteret Lodge Pond, June 28, 1934.

Belostoma sp., juv.

Mullet Pond, June 18, 26, July 11; Open Grounds, ditch, June 20, 1934.

Notonecta irrorata Say

Open Grounds, June 18, 1928**, June 6, 1934*; Harlowe Creek, June 22, 1934*.

**Notonecta* sp., juv.

Mullet Pond, June 18, 1934.

**Mesovelia mulsanti* White

Carteret Lodge Pond, June 28; Mullet Pond, July 11, 1934.

**Trichocorixa verticalis* Fieber

Stream above Morton's Mill Pond, May 1; Mullet Pond, June 18, July 11; Open Grounds, creek, July 2, 1934.

**Trichocorixa* sp., juv.

Mullet Pond, July 11, 1934.

Homopterida

Tibicen chloromera (Walker)

Near Harlowe Creek, June 22; near Eastman's Creek, July 12, 1934.

Orthopterida

Orthopterans were identified by A. N. Caudell (*).

**Hippiscus phoenicopterus* (Burmeister)

Open Grounds, June 20, 1934.

**Homocoryphus malivolans* (Scudder)

A specimen of this species was captured on Piver's Island during the summer of 1934.

**Leptysma marginicollis* (Serville)

Mullet Pond, swamp, June 26, 1934.

**Limnogonus* sp.

Carteret Lodge Pond, June 28, 1934.

**Manomera tenuescens* (Scudder)

Piver's Island, summer of 1934.

**Mermiria* sp.

Lenoxville Road, June 30; Open Grounds, July 2, 3, 1934.

**Oncometopia undata* Fabricius

Harlowe Creek, June 22, 1934.

**Orchelium fidicinium* Rehn & Hebard

Calico Creek, June 21; Lenoxville Road, June 30; Eastman's Creek, July 12, 1934.

**Paroxya clavuliger* (Serville)

Mullet Pond, June 26, Open Grounds, July 2, 3, 1934.

**Pissonotus* sp.

Carteret Lodge Pond, June 28, 1934.

Coleopterida

Beetles were identified by H. S. Barber, A. G. Bovig, C. S. Brimley, L. L. Buchanan, W. S. Fisher, and P. W. Oman.

Berosus infuscatus Lec.

Mullet Pond, July 11, 1934.

Berosus peregrinus Herbst

Carteret Lodge Pond, June 28, 1934.

Canthydrus bicolor Say

Carteret Lodge Pond, June 28, 1934.

Centrinaspis sp.

Morton's Mill Pond, June 19, 1934.

Chauliognathus marginatus (Fabricius)

Morton's Mill Pond, June 19, 1934.

Chrysobothris sp.

Mullet Pond, June 18, 1934.

Cicindela hirticollis Say

Mullet Pond, June 18, 1934.

Cicindela lacerta Chd.

Mullet Pond, June 18, 1934.

Cicindela tortuosa Lec.

Mullet Pond, June 18, July 11, 1934.

Copelatus glyphicus Say

Open Grounds, ditch; June 20, 1934.

Dineutes carolinus Lec.

Open Grounds, stream, July 3; Morton's Mill Pond, July 9, 1934.

Dineutes emarginatus Say

Carteret Lodge Pond, June 20; stream above Morton's Mill Pond, July 9, 1934.

Dineutes serrulatus Leconte

Open Grounds, June 18, 1934.

Enochrus sublongus Fall

Open Grounds, March 28, June 20; Mullet Pond, April 30, 1934.

Enochrus perplexus Lec.

Mullet Pond, April 4, June 18, 26, July 11, 1934.

Galerucella nymphaeae (L.)

Carteret Lodge Pond, June 28, 1934.

Gyrinus analis Say

Open Grounds, ditch, June 18, 1928, June 20, July 2, 1934.

Hydaticus bimarginatus Say

Mullet Pond Swamp, April 30; Eastman's Creek, July 14, 1934.

Hydroporus carolinus Fall

Open Grounds, ditch, June 20, 1934.

Hydroporus clypeatus Sharp

Open Grounds, ditch, June 20; Harlowe Creek, June 22, 1934.

Lixus marginatus Say

Open Grounds, ditch, June 20, 1934.

Megamelus sp.

Carteret Lodge Pond; June 28, 1934.

Melanactes piceus (De G.)

Harlowe Creek, June 22, 1934.

Monochamus titillator Fabricius

Eastman's Creek; July 12, 1934.

Ranthus calidus Fabricius

Open Grounds, June 28, 1934.

Tropisternus glaber Herbst

Open Grounds, June 28, 1928; Mullet Pond, July 11, 1934 (larva).

Topisternus quadristriatus Horn

Open Grounds, pond, March 28; Mullet Pond, March 28, April 1, 30, July 11, 1934.

Dipterida

Dipterous insects were identified by C. S. Brimley, C. T. Greene, G. A. Sandhouse, A. Stone, and the writer.

Aedes canadensis (Theobald)

Core Creek Canal, July 10, 1934.

Aedes sollicitans (Walker)

Open Grounds, ditches and streams, June 20, July 3, 1934.

Anopheles crucians Wiedemann

Open Grounds, June 20, 1934.

Anthrax sinuosa Wiedemann

Morton's Mill Pond; June 19, 1934.

Cerceris fumipennis Say

Morton's Mill Pond, June 19, 1934.

Chaetopsis aenea Wiedemann

Calico Creek, June 21; Eastman's Creek, July 12, 1934.

Chaetopsis fulvifrons Macq.

Stream above Morton's Mill Pond, May 1, June 19. Morton's Mill Pond, June 19; Calico Creek, June 21, 1934.

Chironomus lobiferus Say

Above Morton's Mill Pond, May 1; Calico Creek, May 2; Open Grounds, ditch and stream, June 20, July 3, 1934.

Chironomus flavus Johannsen

Carteret Lodge Pond, June 28, 1934.

Chironomus sp.

Mullet Pond, April 30, June 18, July 11, 1934.

Chrysops vittata floridana Johnson

Harlowe Creek, June 22, 1934.

Diachlorus ferrugineatus Fabricius

Open Grounds, June 20, July 2, 3; Harlowe Creek, June 22; Carteret Lodge Pond, June 28; Morton's Mill Pond, July 9, 1934.

Dolichopodidae

Harlowe Creek, June 22, 1934.

Empidae

Mullet Pond, April 30, 1934.

Ephydra sp.

Mullet Pond, April 30, 1934.

Erax albibarbis.Macq.

Mullet Pond, June 18, 1934.

Hoplodictya spinicornis Lw.

Mullet Pond, April 30, 1934.

Hydrophorus sp.

Open Grounds, March 29; Mullet Pond, April 1, June 18, July 11 (pupal cases), 1934.

Laphystia litoralis Curran

Mullet Pond, June 18, 1934.

Lauxania facialis Coq.

Mullet Pond, April 30, 1934.

Mesogramma polita Say

Calico Creek, June 21, 1934.

Mydas clavatus Drury

Harlowe Creek, June 22, 1934.

Pelastoneurus parvus Aldrich

Mullet Pond, April 30, 1934.

Psorophora ciliata (Fabricius)

Core Creek Canal, July 10, 1934.

Psychodidae

Morton's Mill Pond, June 19, 1934—pupae.

Ptecticus sakeni Williston

Open Grounds, June 28, 1928.

Saldula reperta Uhl.

Mullet Pond, July 11, 1934.

Sarcophaga sarracenioidea Aldrich

Open Grounds, June 18, 1928.

Stratiomyidae

Mullet Pond, April 30, 1934; larvae among algae along shore.

Tabanus costalis Wiedemann

Open Grounds, June 28, 1928.

Tabanus lineola Fabricius

Open Grounds, June 28, 1928.

Tanypus decoloratus Malloch

Open Grounds, June 20; Carteret Lodge Pond, June 28; Mullet Pond, July 10, 1934.

Tipulidae

Along the shores of Mullet Pond on April 30, 1934, crane-fly larvae were common in the sand along shore, and adults in the air. Through an unfortunate accident none was saved for identification.

Merostomata

Xiphosura

Limulus polyphemus (L.)

Beaufort Breakwater, June 13, 1928; Bogue Sound, March 30, Town Marsh Flats, June 27, Mullet Pond, July 11, 1934.

Arachnida

Acarina

Mites were identified by Dr. Ruth Marshall.

Arrhenurus marshallae Piers

Morton's Mill Pond, May 1, 1934 (S:2.0).

Arrhenurus megalurus Marshall

Morton's Mill Pond; June 28, 1934 (S:0.05).

Eylais desecta Koenike

Mullet Pond, swamp; April 1, 30, 1934.

Koenikea concava Wol.

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Koenikea haldemanii Viets

Morton's Mill Pond, June 28, 1934 (S:0.05).

Oribatidae

Morton's Mill Pond, June 28, 1934 (S:0.05).

Araneida

Spiders were identified by Dr. A. M. Chickering.

Dolomedes sp., juv.

Stream above Morton's Mill Pond; Open Grounds, June 20, 1934.

Pardosa pauxilla Montgomery

Mullet Pond, April 30, 1934.

Tetragnatha pallescens Cambridge

Mullet Pond, April 30; above Morton's Mill Pond, May 1, 1934.

Tetragnatha laboriosa Hentz

Swamp above Morton's Mill Pond, May 1, 1934.

Enteropneusta

Balanoglossus aurantiacus (Girard)

Common on the flats near Beaufort; Beaufort Breakwater, July 5, 1928.

Chordata

Piscea

Fishes about which the writer was in doubt were referred to S. F. Hildebrand.

Anguillula rostrata (Lesueur)

Open Grounds, ditch, and stream, June 28, 1928; July 2 (S:0.07); stream above Morton's Mill Pond, May 1 (S:0.73); Harlowe Creek, June 22 (S:0.5); Calico Creek, May 2 (S:0.76), June 21 (S:0.16); Lenoxville Road, June 30, 1934 (S:28.5).

Aphredoderus sayanus (Gilliams)

Open Grounds, ditch; June 28, 1928; June 20 (S:0.5), July 3, 1934 (S:0.07).

Chasmodes bosquinus (Lacépède)

Core Creek, June 14, 1928.

Ctenogobius stigmaticus (Poey)

Harlowe Creek, April 2, 1934 (S:9.27).

Cynoscion regalis (Block & Schneider)

Core Creek Canal, July 10, 1934 (S:22.5).

Cyprinodon variegatus Lacépède

Mullet Pond, June 28, 1928 (S:20.4), June 18 (S:0.05), June 26 (S:12.6), July 11 (S:19.8); Eastman's Creek, July 12, 1934 (S:15.5).

Elassoma zonatum Jordan

Open Grounds, ditch; June 20, 1934 (S:0.3).

Enneacanthus gloriosus (Holbrook)

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Esox americanus (Gmelin)

Open Grounds, ditch, June 18, 1928 (S:0.03).

Eupomotis gibbosus (L.)

Open Grounds, June 18, 1928 (S:0.03); Morton's Mill Pond, April 3, 1934 (S:2.48).

Fundulus diaphanus (Lesueur)

Calico Creek, May 2 (S:0.76), June 21 (S:0.16); Morton's Mill Pond, June 19 (S:5.7); Harlowe Creek, June 22, 1934 (S:0.5).

Fundulus heteroclitus (L.)

Core Creek Inlet, June 14; Fort Macon, marsh, June 27, 1928 (S:34.8); Lenoxville Road, ditch, June 29, 1934 (S:21.4).

Fundulus majalis (Wahlbaum)

Harlowe Creek, April 2 (S:2.2); Calico Creek, July 6 (S:32.0); Core Creek Canal, July 10 (S:21.5); Eastman's Creek, July 12 (S:15.5).

Gambusia holbrooki Girard

Open Grounds, ditch, June 18, 1928, June 20 (S:0.5), July 2, 1934 (S:0.05); Mullet Pond, June 22, 1928 (S:20.4), June 18 (S:0.03), July 11, 1934 (S:0.3–3.2); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19 (S:5.3); Harlowe Creek, April 2 (S:2.16), May 1 (S:0.05); above Morton's Mill Pond, May 1 (S:0.73), July 9; Carteret Lodge Pond, June 28 (S:0.05); Lenoxville Road, ditch, June 30 (33.0); Core Creek Canal, July 10 (S:0.7); Calico Creek, July 6 (S:32.7).

Gobiosoma bosci Lacépède

Mullet Pond, June 26, 1934 (S:12.6).

Hypsoblennius hentz (Lesueur)

Beaufort Breakwater, June 13 (S:32.0); Piver's Island, June 15, 1928 (S:31.3).

Leiostomus xanthurus Lacépède

Common near Beaufort; Core Creek Canal, July 10, 1934 (S:18.9).

Menidia menidia (L.)

Core Creek Canal, July 10, 1934 (S:18.9).

Menidia bryllina (Cope)

Open Grounds, ditch, July 18, 1928 (S:0.5); Mullet Pond, June 18 (S:0.05), June 22 (S:20.2–26.9), June 26 (S:12.6), July 11, 1934 (S: 19.8).

Menticirrhus saxatilis (Bloch & Schneider)

Mullet Pond, June 26, 1934 (S:12.6).

Micropogon undulatus (L.)

The croaker is common in the Beaufort region. Open Grounds, June 18, 1928 (S:0.5); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95); stream above Morton's Mill Pond, May 1 (S:0.73); Core Creek Canal, July 10 (S:25.8).

Micropterus salmoides (Lacépède)

Open Grounds, ditch, June 18, 1928.

Mugil cephalus L.

Core Creek, July 10, 1934 (S:25.8).

Mugil curema Cuvier & Valenciennes

Morton's Mill Pond, April 3 (S:2.48); Calico Creek, July 6, 1934 (S:34.1).

Notemigonus chrysoleucas (Mitchill)

Open Grounds, ditch, June 18, 1928.

Notropis hudsonius (Clinton)

Open Grounds, ditch, June 18, 1928.

Opsanus tau (L.)

The toadfish is common near Piver's Island and elsewhere in the Beaufort region.

Orthopristis chrysopterus (L.)

Core Creek, June 25; Mullet Pond, June 26 (S:12.6); Core Creek Canal, July 10, 1934 (S:25.8).

Paralichthys dentatus (L.)

Open Grounds, July 18, 1928; Morton's Mill Pond, June 19, 1934 (S:5.3).

Pteroplatea maclura (Schneider)

Core Creek Inlet, June 16, 1928.

Spheroides maculatus Block & Schneider

Beaufort Breakwater, June 25, 1928.

Symphurus plagiusa (L.)

Core Creek Canal, June 25, July 10, 1934.

Amphibea

Frogs and toads were identified by B. B. Brandt.

Acris gryllus (Le Conte)

Open Grounds, March 29; Carteret Lodge Pond, June 28, 1934.

Bufo fowleri Garman

Open Grounds, June 20, 1934.

Hyla squirella Latreille

Mullet Pond, swamp, June 26 (S:0.4).

Rana catesbeiana Shaw

Carteret Lodge Pond, June 28, 1934.

Rana sphenocephala (Cope)

Mullet Pond, June 18 (S:0.3), June 26 (S:0.4); Morton's Mill Pond (S:4.9); Harlowe Creek, June 22 (S:3.5–0.5); Open Grounds, March 29 (S:0.3), July 2 (S:0.0), July 3 (0.07); Calico Creek, June 21 (S:0.16); Carteret Lodge Pond, June 28 (S:0.05); Lenoxville Road, ditch, June 30 (S:21.4); Core Creek Canal, July 10, 1934 (S:0.7).

Reptilea

Alligator mississippiensis (Daudin)

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Anolis carolinensis Cuvier

Near Mullet Pond, July 21, 1928, June 18, Carteret Lodge Pond, June 28, 1934.

Caretta caretta (L.)

Loggerhead turtles are often taken by fishermen in the sounds near Beaufort.

Chelone mydas (L.)

Green turtles are often captured in the sounds near Beaufort.

Chelopus guttatus (Schneider)

Open Grounds, June 20, 1934 (S:0.0).

Chelydra serpentina (L.)

Mullet Pond, Swamp, July 11, 1934 (S:0.3).

Kinosternon subrubrum (Lacépède)

Open Grounds, March 28, June 21, July 3; Mullet Pond, Swamp, June 18; Harlowe Creek, June 22, 1934.

Avea

Many birds were observed, but no record was kept of their occurrence. Coues and Yarrow (1878) give a list of the birds they observed in the Beaufort region.

Mammalea

Prodelphinus plagiodon (Cope)

Porpoises ranged through the sounds near Beaufort, but were never seen in the streams.

LIST OF PLANTS OBSERVED

Thallophyta

Hoyt (1921) has published a list of the algae of the Beaufort region.

Enteromorpha intestinalis (L.) Grev.

Mullet Pond, April 30 (S:9.11); Calico Creek, May 2, 1934 (S:0.76).

Microspora sp.

Open Grounds, pond; March 28, 1934 (S:0.03).

Ulva lactula L.

Adams Creek, April 2, 1934 (S:0.03).

Bryophyta

Sphagnum sp.

Open Grounds, pond, March 28 (S:0.03), June 20 (S:0.3).

Spermatophyta

Juncus raemerianus Scheele

This sedge was common on swampy areas along brackish inlets. Above Morton's Mill Pond, May 1 (S:0.73), July 9, 1934 (S:12.64–18.23).

Sabal glabra (Mill.) Sarg.

Dwarf palmettos often occur along brackish water areas near Beaufort. Open Grounds, stream, July 3 (S:0.07); Core Creek Canal, July 6, 1934 (S:18.9).

Salicornia perennis Mill

Eastman Creek, July 12, 1934; common on flats (S:27.7).

Typha angustifolia L.

Open Grounds, ditch, March 20 (S:0.00); Mullet Pond, Swamp, June 18 (S:0.03); along stream above Morton's Mill Pond, July 9, 1934 (S:18.2).

TOLERATION OF MARINE AND ESTUARINE ANIMALS TO VARIATIONS IN SALINITY

During the summer of 1928 Miss Sophie Dehler carried on a series of experiments for the writer to determine the ability of littoral marine

TABLE 1

Average time in hours that various animals lived in fresh water, sea water, and in various mixtures of the two

+ after a number indicates that animals were alive and in good condition when an experiment was discontinued.

ANIMAL	NUMBER USED	FRESH	1:3	1:1	3:1	SEA WATER
Annelid:						
Chaetopterus pergamentaceus Cuv.	20	10.5	10.5	16.5	28.5	28.5
Ophiuroid:						
Ophiura brevispina Say	35	16	20.5	35	83+	83+
Echinoids:						
Arabacia punctulata (Lamarck)	10	14.5	14.5	22	64	51
Lytechinus variegatus (Lamarck)	30	16	16	16.5	35	71
Mellita quinquiesperforata (Clark)	20	8	26	56.5	68.5	436+
Snails:						
Busycon p. perversum (L.)	10	17	42	60	606+	1095+
Crepidula fornicata (Verrill)	10	20.5	20.5	33	33	33
Fasciolaria tulipa L.	25	8	8	14	30	31
Nassarius obsoleta Say	15	42	42	1124+	1124+	1124+
Oliva s. sayana Ravenel	18	15	32.5	121+	199+	221+
Polynices duplicata Say	24	63	69	140+	194+	264+
Strombus p. pugilis L.	9	16	24	119	214+	253+
Terebra dislocata Say	40	15.5	19	476+	595+	639+
Urosalpinx cinereus (Say)	30	20	28	796+	796+	796+
Clams:						
Bankia gouldi Bartsch	14	15	106+	106+	117+	23
Cardium muricatum L.	10	15	15	15	746	984+
Chione cancellata L.	20	85	102	157	174	736+
Dosinia discus Reeve	10	54	102	308	284	114
Lucina filosa Stimpson	10	27	125	196+	281+	281+
Spissula solidissima similis Say	12	15	32	48	96	159
Modiolus d. demissus Dillwyn	10	148	330+	330+	330+	330+
Ostrea virginica Gmelin	13	70	70.5	288+	271+	382+
Pecten i. irradians Lamarck	25	8	8	14	30	31
Sinum perspectivum Say	20	24.5	33	106+	151	110
Solemya velum Say	80	20	22	32	218	190
Tagelus gibbus Spengler	20	16	37	40	53	41
Tellina alternata Say	4	15	21	21	21	—
Venus m. mercenaria L.	20	114+	102+	120+	120+	120+
Barnacle:						
Balanus eburneus Gould	12	24.5	63	164	305	305
Amphipod:						
Orchestia platensis Kroyer	22	1	14	270	172	16

TABLE 1—*Concluded*

ANIMAL	NUMBER USED	FRESH	1:3	1:1	3:1	SEA WATER
Hermits, Crabs, etc.:						
Clibanarius vittatus (Bosc)	10	20	432+	432+	432+	432+
Emerita talpoidea (Say)	20	26	11	21	206	14.5
Eurypanopeus depressus (Smith)	10	27.5	904+	892+	910+	910+
Pagurus longicarpus Say	10	20	240+	432+	432+	432+
Panopeus herbstii H. Milne-Edw.	10	21.5	504+	504+	504+	504+
Pinnixa chaetopterana Stimpson	10	4.5	7	43	408	408
Polyonyx macrocheles (Gibbes)	71	11	11	11	138+ 96	214+ 165+
Sesarma cinerea Say	10	87	289	780+	713+	181
Uca pugnax (Smith)	25	28	1213+	1059+	1114+	1052+
Xiphosuran:						
Limulus polyphemus (L.)	20	38	279+	714+	775+	714+
Tunicate:						
Molgula manhattensis (De Kay)	10	14	23	23	31	62
Fishes:						
Fundulus heteroclitus (L.)	10	598+	317	401	408	271
Fundulus majalis (Lesueur)	17	12	252	267	283	284
Lagodon rhomboides (L.)	5	1	1.5	1	16.5	16.5
Menidia bryllina (Cope)	5	0.5	1	64	16	40

and estuarine animals to live in dilutions of sea water. The work was done between June 10 and August 31. Temperatures in the dishes where marine animals were kept in the laboratory varied between 22.3°C. and 29.6°C. Salinities of the running sea water supplied in the laboratory varied between 32% and 38% (Gutsell 1931). The results of Miss Dehler's tests are shown in Table 1. Perhaps the deaths of some of the animals were due to the conditions of the experiments, but it is perhaps significant that none lived longer in fresh water than in dilutions of sea water. Of the forty-five specimens tested only eight lived longer in brackish water than in undiluted sea water. These were (1) Bankia, Modiolus, and Ostrea (which are typical brackish water animals) (2) Orchestia and Sesarma (which are terrestrial animals that live near the sea), *Fundulus heteroclitus* (a fish which migrates freely from salt to fresh water) and Emerita (a burrowing crustacean which lives on sandy bottoms where there are strong currents and waves).

Among the animals that were found in localities, such as Mullet Pond, Harlowe Creek, Calico Creek, and Morton's Mill Pond, where

salinities were continually fluctuating and often low, the following may be mentioned:

Morton's Mill Pond (S:2.48–20.6): Marine types, such as blue crabs, *Palaemonetes carolinus*, *Peneaus brasiliensis*, Tellina, Corophium, Leptochelia, Orchestia, *Nereis limbata*, Rhythropanopeus, Elysia, mussels, *Balanus eburneus*, *Uca minax*, *Gammarus locusta*, Mugil, Micropogon, Nemopsis, flounder, and Dactylometra, were associated with fresh water types of chironomid larvae, Gambusia, Carinogammarus, Polymesoda, *Fundulus diaphanus*, and *Macrocyclops albidus*.

Calico Creek (S:0.16–20.0): with Paludestrina, Littoridina, *Littorina irrorata*, *Nereis limbata*, Sesarma, *Uca minax*, *Callinectes sapidus*, *Palaemonetes carolinus*, *Peneaus brasiliensis*, and *Mytilus exustus* were found Gambusia, *Fundulus diaphanus*, eel, several odonates, *Chironomus lobiferus*, and *Microcyclops albidus*.

Harlowe Creek (S:0.00–26.0): with *Uca minax*, Sesarma, *Callinectes sapidus*, *Peneaus brasiliensis* and *P. setiferus*, *Palaemonetes carolinus*, *Balanus eburneus*, and *Ostrea virginica* lived Polymesoda, Gambusia, chironomid larvae, bugs, flies, leopard frog, and eels.

Mullet Pond (S:8.3–13.1) with Enteromorpha, Callinectes, *Uca pugilator*, Orchestia, *Palaemonetes carolinus*, Erichsonella, Leptochelia, Tellina, Littoridina, *Crepidula fornicata*, *Leptonereis culveri*, *Glycera americana*, Myxobdella, Edwardsia, Rotalia, Menidia, Cyprinidon, Menticirrus, Orthopristia, lived a number of species of beetles, dragonflies, damselflies, bugs, and dipteridans.

Open Grounds (0.0–03): the blue crab, Rithropanopeus, *Uca minax*, *Balanus improvisus*, *B. eburneus*, *Peneaus brasiliensis*, *Palaemonetes carolinus*, and *Menidia bryllana* were associated with crayfishes, Gambusia, Esox, Eupomotis, Aphredoderus, Anguilla, Notemigonus, Natrix, Rana (and tadpoles), Kinosternon, Succinea, and a variety of insects (beetles; damsel-, dragon-, and may-flies; and bugs).

Arthropods and fishes are the types that best tolerate extreme variations in salinity. When sea water becomes extremely dilute a few marine types of crustaceans and fishes persist; when the sea water is diluted only slightly with fresh water representatives of comparatively specialized orders (Coleopterida, Dipterida) and of some more primitive orders (Odonatida, Heteropterida) are the first to enter it.

TOLERATION OF DESICCATION BY ESTUARINE ANIMALS

During the summer of 1928 Miss Dehler tested the ability of certain estuarine animals to endure desiccation. Animals were placed in open,

TABLE 2

Length of time in hours that certain estuarine animals live when exposed to desiccation in air; June, July 1928

SPECIES	NUMBER TESTED	AVERAGE	MAXIMUM
Anemone:			
Sagartia luciae (Verrill)	10	25.8	28.0
Echinoderms:			
Ophiura brevispina (Say)	11	3.1	4.3
Arbacia punctulata (Lamarck)	10	47.0	47.0
Snails:			
Fasciolaria tulipa L.	10	72.8	91.1
Littorina irrorata Say	10	184+	184+
Nassarius obsoleta Say	10	58.3	64.8
Urosalpinx cinereus (Say)	10	44.3	57.5
Clams:			
Chione cancellata L.	10	49.2	66.0
Modiolus d. demissus Dillwyn	10	209+	209+
Ostrea virginica Gmelin	31	115.1	210.1
Pecten i. irradians Lamarck	11	18.8	18.9
Solemya velum Say	10	19.0	19.0
Venus mercenaria L.	10	255.8	310.1
Barnacle:			
Balanus eburneus Gould	10	82.5	93.0
Hermit crabs:			
Clibanarius vittatus (Bosc)	10	141.0	281.0
Pagurus longicarpus Say	10	19.9	40+
Shrimp:			
Palaemonetes carolinus Stimpson	10	4.8	5.0
Crabs:			
Callinectes sapidus Rathbun	14	34.4	97.3
Eurypanopeus depressus (Smith)	10	36.8	44.3
Libinia dubia H. Milne-Edw.	1	22.8	—
Ocypode albicans Bosc	10	87.5	115.1
Panopeus sayi Smith	10	47.3	67.0
Sesarma cinerea Say	10	32.8	46.8
Uca minax (Le Conte)	9	42.7	51.3
Uca pugilator (Bosc)	10	40.2	40.2
Isopods:			
Ligyda oceanica (L.)	10	27.8	42.3
Orchestia platensis Kroyer	10	9.4	9.4
Xiphosuran:			
Limulus polyphemus (L.)	10	63.5+	63.5+

clean glass dishes and allowed to stand on laboratory tables. No determinations of humidity, temperatures, or air currents were made. The results of these tests are shown in Table 2. The types which showed

most resistance were those which were accustomed to spend much time out of water (Littorina), and those with heavy protective shells (Limulus, Venus, Clibanarius). In general larger animals survived longer periods of desiccation than smaller. Small arthropods in general showed little resistance (Orchestia, Palaemonetes).

DISCUSSION

According to Redeke (1922) brackish water animals in Holland may be placed in three groups: (1) *Oligohaline*, which have been largely derived from fresh water and usually live in salinities between 0.2 and 1.9%; (2) *Mesohaline*, which include many proper brackish water animals which live in salinities between 2.0 and 18.5%; and (3) *Polyhaline*, which are mostly marine and live in salinities between 18.6 and 31.8%. Remane (1934) found that fewest species lived at the borderline where fresh water and marine animals met, at salinities of 5–8%. In his paper on the marine molluscs of the Beaufort region Jacot (1921) points out that there are two groups in regard to distribution: (1) the species that occur on the beach of the open sea and (2) those in the sounds. The former seldom get into the sounds and many of them occur in deep water.

In the experiments described in the present paper in which the ability of various estuarine animals to live in diluted sea water was tested (Table 1) none was found which lived in fresh water longer than in sea water. About seven seemed to be better fitted for brackish water than for either fresh or sea water. Perhaps these are to be looked upon as proper brackish water types. Two of them (Sesarma, Orchestia) are really burrowing land animals that live at the drift line above high-tide mark, but neither can exist away from the ocean. Three of the others (Bankia, Modiolus, Emerita) are burrowers which usually remain submerged and are only active under water. Another, the oyster, is a sessile animal which is also active only when submerged, and is, as many investigators have shown (Amemiya 1928), adapted to brackish water. The seventh species, *Fundulus heteroclitus*, is an euryhaline type which migrates freely from fresh to salt water, and will survive direct transfer from the sea to fresh water (Sumner 1911). As Annandale (1922) has pointed out many types of marine animals continually attempt to spread from the ocean into estuaries, but comparatively few become permanently established.

Many estuarine types are adapted to live in the silty deposits which are characteristic on bottoms. Many burrow and are able to tolerate

very low oxygen pressures in the water which surrounds them (Cole 1926). Life in the soft bottom has certain advantages: the water in the floor of an estuary often remains more salty than that above (Alexander, Southgate, and Bassindale 1932) when water is flowing out from the fresh water tributaries and oxygen in burrows may at times be maintained at a rather high concentration by seepage (MacGinitie 1935).

Typically marine animals which have crossed the brackish regions of the estuaries and almost or actually entered the fresh waters beyond are types like (1) oysters and barnacles, which are so well insulated by thick calcareous coverings that they can fast for long periods during freshets and feed only when salinities are favorable, and (2) active, euryhaline types such as the blue crab (Callinectes) and the top minnow (*Fundulus heteroclitus*). There are certain obstacles which retard or prevent the entrance of marine animals into fresh water. Respiration is more difficult in fresh water (Schlieper 1933). Calcium is essential for certain animals, probably in connection with buffer actions and alkali reserves, and when present in quantity certain fishes can leave the ocean and enter waters that contain it (Breder 1933). Such things as barnacles and oysters may by favorable combinations of circumstances become established and with their rather remarkable powers maintain themselves and grow for a time, but they can probably never become fresh water animals. The inability to carry on successful reproduction is probably the final factor which prevents many marine animals which almost succeed from attaining fresh water permanently.

If one looks in the Beaufort estuaries for types of animals that are leaving land and fresh water to enter the ocean there are few to be found. No mollusc appears to be migrating from fresh water to sea, though some are favorably situated (Polymesoda; van der Schalie 1933). Insects are continually carried down by streams into estuaries and some have become established in the latter, but they are few in numbers compared with those which are found in brackish ponds and marshes. This is probably because insects, though generally progressive and aggressive, are strongly committed to air-breathing through tracheal tubes; and open sounds with strong waves and currents are therefore unfavorable. Insects are invading the sea from land and fresh water (Pearse 1932, 1936) but are to be found in quiet, protected waters. They are favored in this by the fact that ponds and marshes, even quite near to the ocean, often tend to maintain salinities much below that of sea water. In such habitats insects are often associated with typically marine animals.

Many animals appear to have crossed ocean beaches to become

established gradually on land, but few have attained fresh water or land through estuaries (Annandale, 1922; MacGinitie, 1935; Pearse 1936).

SUMMARY

1. Beaufort, North Carolina, is favorably located for the study of estuarine animals, as it is surrounded by banks, sounds, and estuaries.

2. During two summers collecting was carried on and determinations of salinity, temperature, oxygen, and hydrogen-ion concentration were made. Tides at Beaufort usually have a vertical range of 2.5 ft.

3. A list of animals and a few plants collected is given.

4. The toleration of 45 estuarine species for dilutions of sea water was tested. Among these none lived longer in fresh water than in sea water, seven appeared to be adapted to brackish water, and the remainder were marine.

5. The toleration of 25 estuarine animals to desiccation was tested. Types which proved to be most resistant were those accustomed to spend much time out of water and those with thick protective shells.

6. Few marine animals appear to have migrated through estuaries to attain life on land, but many have gone from the ocean directly over beaches. Difficulties encountered in estuaries are concerned chiefly with salinity, respiration, and reproduction.

7. Some estuarine animals show adaptations for burrowing and toleration of low oxygen concentrations; others are active and euryhaline.

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PLATE 15

FIG. 1. POND IN PEAT; OPEN GROUNDS
FIG. 2. HARLOWE CREEK CANAL

FIG. 3. MULLET POND
FIG. 4. ALONG LENOXVILLE ROAD

RACIATION IN STENIRIDIA ANDREWSI HARRIS, A SUPPLEMENT TO SPECIATION IN STENIRIDIA

By J. M. Valentine

Plate 17

It has been very tactfully and pleasantly brought to the author's attention by his colleagues that he has "committed a synonym." In his recent paper, *Speciation in Steniridia*,* he has unwittingly placed Casey's *Steniridia amplicollis* (1920) and *reflexa* (1924) as variants of *Steniridia andrewsi montana*, new subspecies. The excuse, admittedly a poor one, is that arbitrary taxonomy was for the time obscured by much more significant biological data. Though the author rebels, on pragmatic grounds, against the obligation of accepting an aberrant individual as the type of an important and widely ranging geographical race, he finds it his painful duty to compromise in order to comply with a necessary rule of nomenclatorial priority.

Amplicollis obviously does not typify the great *andrewsi* population inhabiting the mountains of northwestern North Carolina and southwestern Virginia, which race, for the sake of simplicity, the writer proposed (1935) to embrace with all its possible subraces under one name —*montana*. Casey's type specimen would cause a never-ending source of annoyance and false hope among future students who, comparing their more normal examples of *andrewsi* with it, might easily be tempted to describe, in the Caseyan manner, any number of "new" things within the species. Rather than run this risk, it seems better to split the existing subspecies of *andrewsi* into a secondary series of races, thus repeating the analysis of the species at a level of greater dichotomy. A certain degree of simplicity will doubtless be sacrificed by this process, yet at the same time a more complete picture will be gained of the relationship between genetic trends and the topographic features which act as barriers to and vehicles of distribution.

In *montana*, there is every gradation in pronotal contour from the straight-sided, narrowly margined pronotum close to that of race *germari*, to the condition of widely flaring rounded margins seen in

* See bibliography.

amplicollis, an extreme case. It is true, however, that the widest margins are typical of examples from the somewhat isolated Black Mountain Range of North Carolina. It is likewise true that narrower margins are characteristic of the *andrewsi* fauna from the type locality of *montana* and from the adjoining Grandfather Mountain, Avery Co., North Carolina. Given the existing type material, these subraces should be differentiated. If *they* are subspecifically handled, it will be necessary to undertake a parallel splitting within other forms of *andrewsi* already described as subspecies. Thus *germari* and *parvitarsalis* must also undergo geographico-genetic subdivision. To accomplish this, other external characters such as general proportions, color, sculpture and male tarsal scalation must be brought in to supplement the variable pronotal contour.

A knowledge of relatively exact localities is a primary prerequisite to proper identification of geographic races; for this reason it seems unnecessary and misleading to adopt the key method of separating such forms. We are dealing with freely crossing segregates belonging to *one genitalically constant species* whose external characters vary with locality and environment; we are not herein concerned with stable, true-breeding species of drastically distinct genitalic equipment.

Additional data acquired subsequent to the publication of *Speciation in Steniridia** are included in the following revision of *Steniridia andrewsi*. The same technique in measurement and in calculating indices of parts will be followed here. For details and explanations the reader is referred to the first paper.

Steniridia andrewsi may now be divided into the following groups:

I. *Group andrewsi* s. str.: Relatively large, brilliant forms with comparatively smooth sculpture, narrow, much reflexed pronotal margins and restricted development of the scaly pads on the male anterior tarsi (tarsal index not over .53).
Chorology: Low altitudes; piedmont section of central North Carolina.
1. *Steniridia andrewsi andrewsi* Harris.

II. *Group montana:* Medium sized, relatively dark forms with deeper, more irregular sculpture, wider pronotal margins and in the male the most extensive anterior tarsal pad development (tarsal index .69–.82).
Chorology: Intermediate to fairly high altitudes, mainly the

* See bibliography.

former; the Appalachian Mountains from southwestern Virginia southwest through North Carolina and along the Tennessee line to the French Broad River.

2. *Steniridia andrewsi montana* Valentine.
3. *Steniridia andrewsi amplicollis* Casey.

III. *Group parvitarsalis:* Similar to the last but with the plantar pads of the male front tarsus reduced in size (tarsal index .60–.69).

Chorology: Intermediate to high altitudes, mainly the former; the extreme southwestern end of the Appalachian Mountains: in North Carolina, west of the French Broad River and south of the river system of the Little Tennessee; in South Carolina, Georgia and Tennessee, those mountainous portions immediately adjacent to the above area in North Carolina.

4. *Steniridia andrewsi parvitarsalis* Valentine.
5. *Steniridia andrewsi nantahalae* n. ssp.
6. *Steniridia andrewsi saludae* n. ssp.

IV. *Group darlingtoni:* Medium to small, very dark, deeply, iregularly sculptured races of variable form and tarsal pad development (tarsal index .62–.70).

Chorology: Intermediate to very high altitudes in the Smoky Mountains of North Carolina and Tennessee north of the river system of the little Tennessee.

7. *Steniridia andrewsi darlingtoni* Valentine.
8. *Steniridia andrewsi barksdalei* n. ssp.

V. *Group germari:* Medium to large, relatively brilliant forms having locally irregular sculpture, narrowly margined, straightsided (posterior to lateral angle) pronota and reduced male tarsal pad development (tarsal index .47–.69).

Chorology: Low to intermediate altitudes; within the Appalachian Plateau over that area south of the glacial line drained by the Ohio and Tennessee Rivers and their tributaries.

9. *Steniridia andrewsi germari* Chaudoir.
10. *Steniridia andrewsi mutabilis* Casey.
11. *Steniridia andrewsi waldensia* Valentine.

1. **Steniridia andrewsi andrewsi** Harris

Cychrus andrewsii Harris, 1839

Irichroa andrewsi Harris—Casey, 1920

Steniridia andrewsi Harris—Casey, 1924

Type: ♀, lost.
Neotype: ♀, U. S. National Museum; J. M. Valentine, collector, 1934.
Neotype locality: Chapel Hill, Orange Co., North Carolina.

An additional record from Climax, Guilford Co., North Carolina, extends the range of the type race forty miles to the west.

2. **Steniridia andrewsi montana** Valentine, 1935

Holotype, allotype: ♂, ♀, U. S. National Museum; J. M. Valentine, collector, 1934.
Type locality: Beech Mountain, Avery Co., North Carolina.

Among the males of this race are found individuals exhibiting a greater degree of anterior tarsal dilation and pad development than is characteristic of any other known *Steniridia* (tarsal index .70–.82).

The range includes the mountains of northwestern North Carolina (Avery, Watauga Cos.) and probably the extension of the same high mountain chain in southwestern Virginia (Grayson Co). Examples from further north at Mountain Lake, Giles Co., Virginia, possess pronota which, in their narrowness, tend toward some race of the *germari* group. However, the male tarsi (index .70–.77) are of the *montana* type; for this reason the colony had best be included in *montana* whose range doubtless overlaps that of a subrace of *mutabilis* along the New River. A single female, taken by the author at Little Switzerland, McDowell Co., North Carolina, establishes the southernmost record for the race.

3. **Steniridia andrewsi amplicollis** Casey

Irichroa violacea amplicollis Casey, 1920

Irichroa andrewsi reflexa Casey, 1924

Steniridia andrewsi montana Valentine, 1935

Steniridia andrewsi montana var. *amplicollis*

Casey—Valentine, 1935

Type, paratype: ♂, ♀, U. S. National Museum; Wm. Beutenmuller, collector, 1912.
Type locality: Black Mountains, North Carolina.

Type ♂: length 23 mm.; width 8.8 mm.; head index .45; pronotal index .78; elytral index .68; abdominal index .42; tarsal index .715. *Color:* violaceous above with faint blue reflections.

Topotypes, Black Mountains and Mt. Mitchell, Buncombe and Yancey Cos., North Carolina: seven ♂s: length 20.5–22.2 mm.; width 7.6–8.6 mm.; head index .43–.46; pronotal index .76–.83; elytral index .66–.72; abdominal index .42–.49; tarsal index .69–.79. Four ♀s: length 22.7–23.6 mm.; width 8.9–9.5 mm.; head index .42–.44; pronotal index .78–.80; elytral index .67–.75; abdominal index .42–.47. *Color:* violaceous above with variable blue reflections, less evident on elytra; below black, epipleurae very faintly violaceous; tarsi dark brown.

The type specimen of *amplicollis* is an exaggerated form of its race having pronotal margins of probably maximum extent. The paratype is more normal, being essentially similar to the above topotypes. *Amplicollis,* more or less isolated on the Black Mountain Range, has departed from its closest relative, *montana,* in the following overlapping characters: the color is more violet and less blue; the pronotum is more broadly margined, especially at the rounded lateral angles, and possesses on the average fewer basal punctures, often lacking them entirely; the male tarsi have, on the average, smaller first plantar pads.

Andrewsi refleza Casey (Plate 17, fig. 1), likewise from the Black Mountains, is merely a large (24.6 mm.) female of *amplicollis,* identical in all other respects with topotype material of the latter.

Concerning the habits of *amplicollis,* Beutenmuller (1918) writes: "I have never taken it on all my trips on the extreme summit or higher parts of the slopes."

4. **Steniridia andrewsi parvitarsalis** Valentine, 1935

Type: ♂, U. S. National Museum.
Type Locality: Clayton, Rabun Co., Georgia.

With the separation of *nantahalae* from *parvitarsalis,* the range of the latter race is now restricted to the mountains chiefly of Hiwassee and Little Tennessee drainage including the Blue Ridge of northeastern Georgia and the Unaka Mountains of southwestern North Carolina and southeastern Tennessee. It lives in the relatively low mountains flanking the main Appalachian chains.

5. **Steniridia andrewsi nantahalae** Valentine, n. ssp.

Steniridia andrewsi parvitarsalis Valentine, 1935

Type: ♂, U. S. National Museum; J. M. Valentine, collector, 1931.
Type locality: Cashier's, Jackson Co., North Carolina; 3500 ft.

Type ♂: length 20.0 mm.; width 8.1 mm.; head index .45; pronotal index .80; elytral index .72; abdominal index .47; tarsal index .69. *Color:* above, dark violaceous with blue reflections especially on the margins; below, black, epipleurae faintly purple; tarsi brown, not dark. *Head:* normal. *Pronotum:* margins widely flaring anterior to obtuse but evident lateral angles; elongated, straight-sided and tapering posterior to lateral angles; lateral and anterior margins and basal impressions marked with confused punctures. *Elytra:* normal in form; discal costae interrupted half way to base, obliterated over apical fourth (Plate 17, fig. 2).

Paratype: ♂, Jocassee, Oconee Co., South Carolina; Whitewater River 2200 ft.; D. Dunavan, collector. Length 19.8 mm.; width 7.9 mm.; head index .46; pronotal index .85; elytral index .72; abdominal index .48; tarsal index .69. *Color*: above, dark violaceous.

The high, southern Blue Ridge Mountains at the point where they are joined by the Nantahala and Cowee Mountains are the home of typical *nantahalae.* Being a high altitude form of *parvitarsalis*, it differs from the latter chiefly in those characters which are correlated with elevation: it is smaller, darker, and exhibits a more extensive interruption of elytral costae. However, the greater development of the male's anterior plantar pads is a character which probably is not so closely related to environment but serves rather to connect, genetically, the two groups: *parvitarsalis* and *montana.*

A series collected by G. Beyer in the "Blue Ridge Mountains, North Carolina" and measured by the author (see bibliography) is identical with the type. Another series of three females taken at Rocky Bottom, Pickens Co., South Carolina, by D. Dunavan and O. L. Cartwright agrees with the type in all respects save that these examples have a more angular pronotum and are of the size of *parvitarsalis*: length 21.5–23.2 mm.; width 8.3–9.4 mm.; head index .42–.46; pronotal index .84–.89; elytral index .69–.72; abdominal index .47–.51. A single female taken at Sunburst, Haywood Co., North Carolina, by O. L. Cartwright represents the Pisgah Ridge segregate of *nantahalae:* length 20.7 mm.; width 8.0 mm.; head index .48; pronotal index .82; elytral

index .69; abdominal index .46. The lateral margins of the pronotum of this specimen also are more angular than in the type.

6. **Steniridia andrewsi saludae** n. ssp.

Type: ♀, U. S. National Museum: W. F. Fiske, collector, 1903.
Type locality: Melrose, Polk Co., North Carolina.

Type ♀*:* length 20.8 mm.; width 8.4 mm.; head index .42; pronotal index .87; elytral index .73; abdominal index .50. *Color:* above, dark violaceous; below, black, epipleurae faintly purple; legs dark brown (may be due to slight immaturity), tarsi not perceptibly lighter. *Head:* normal. *Pronotum:* narrow, straight-sided posterior to rounded lateral angles; margin narrow, abruptly reflexed especially in posterior half; impunctate except in basal impressions. *Elytra:* conspicuously elevated in the humeral region, humeri prominently rounded; costae pronounced, interrupted only over apical fourth of elytra; striae deeply, regularly punctured; epipleurae coarsely, irregularly and profusely punctured (Plate 17, fig. 3).

Until males are found, *saludae* is tentatively included in group *parvitarsalis* because of its geographic proximity to members of the latter. However, it bears an equal resemblance to the piedmont-dwelling, neotypic race of *andrewsi* from which it differs thus: it is smaller, darker (including tarsi) and more convex; its pronotum is narrower, the humeri more expanded and the elytral punctation coarser throughout. All these are characters which may simply reflect life at higher elevations in the Saluda Mountains where it was taken.

The Saluda Mountains constitute the eastern extremity of the southern Blue Ridge from the main range of which they are almost completely separated by the French Broad river system.

7. **Steniridia andrewsi darlingtoni** Valentine, 1935

Holotype, allotype: ♂, ♀, Museum of Comparative Zoology; P. J. Darlington, Jr., collector, 1930.
Type locality: State Road to Newfound Gap, Sevier Co., Tennessee; 3500 ft.

Darlingtoni seems to be typical of the high mountains of the southwestern end of the Great Smoky Range along the North Carolina–Tennessee line. Here it ascends to the summit cloud forests of spruce and balsam. Under these ecological conditions are found the smallest,

darkest specimens of roughest sculpture and narrowest pronotal margination. A female, collected by L. Barksdale from the divide running at an elevation of about 4000 ft. from Andrews Bald to Mt. Buckley, has the following characters: length 19.5 mm.; width 7.6 mm.; head index .42; pronotal index .91; elytral index .69; abdominal index .47. *Color:* head and pronotum blue-green-black; elytra violaceous-black; tarsi almost black. The pronotum is very narrow and a lateral band of vermicular costal interruption extends to within one third of the distance to the elytral base.

8. **Steniridia andrewsi barksdalei** n. ssp.

Holotype, allotype: ♂, ♀, U. S. National Museum; L. Barksdale, collector, 1934.

Type locality: Mt. Guyot, Swain and Haywood Cos., North Carolina; about 3500 ft.

Holotype ♂: length 20.9 mm.; width 8.1 mm.; head index .44; pronotal index .78; elytral index .71; abdominal index .44; tarsal index .69. *Color:* above, dark blue with violaceous reflections; below, black, epipleurae faintly violet; tarsi dark brown, anterior ones paler. *Head:* median clypeal groove deeply impressed. *Pronotum:* anterior lateral margins broadly, evenly rounded, lateral angles almost obliterated, posterior lateral contour slightly concave; margins and basal disc impressed with shallow, confused punctures. *Elytra:* humeri reduced; sculpture coarse but regular, punctation wide, deep, causing costae to appear distinctly wavy; discal costae locally vermiculate over apical half, interrupted over apical fifth (Plate 17, fig. 4).

Allotype ♀: length 23.2 mm.; width 9.0 mm.; head index .44; pronotal index .81; elytral index .69; abdominal index .45. *Color:* similar to type but more violaceous; all tarsi dark brown. In body form and sculpture it is identical with the type.

This race seems to hold an intermediate position between *nantahalae* and more northerly races of *andrewsi*. The deeply pitted elytra of beaded appearance and more extensively scaled male front tarsus serve to distinguish it from the former while sculpture, particularly that of the pronotum, and pronotal contour are the principle features differentiating it from group *montana*.

A geographical connection with *nantahalae* probably exists along the Balsam Mountain ridge which joins the northeastern end of the Smoky Mountains, where *barksdalei* is found, with the Tennessee and Pisgah Ridges of the southern Blue Ridge system.

It is a pleasure to name the subspecies after Lane Barksdale who collected the only two specimens seen by the author.

9. **Steniridia andrewsi germari** Chaudoir, 1861

Type: ♀, collection of R. Oberthür-Rennes.
Type locality: Tennessee.

Specimens from Pineville, Bell Co., in the extreme southeastern corner of Kentucky, in all likelihood represent typical *germari* since this locality lies in the Cumberland Mountains which extend well into Tennessee. A much larger race, *waldensia*, inhabits the Walden Ridge to the south. All other important mountain ranges in eastern Tennessee are represented in the author's collection by examples of *andrewsi*, none of which corresponds to the original description of *germari*. It would seem probable, therefore, that somewhere in the Cumberland Mountains of Tennessee lies the true type locality of this insect. Added evidence in support of this view is that specimens from both the Kentucky and Virginia portions of the Cumberlands agree perfectly with Chaudoir's description.

A diagnosis of the Pineville specimens follows: ♂: length 20.5 mm.; width 7.9 mm.; head index .45; pronotal index .86; elytral index .71; abdominal index .45; tarsal index .58. ♀: length 24.4 mm.; width 9.8 mm.; head index .44; pronotal index .87; elytral index .73; abdominal index .47. *Color* of both: violaceous above, head and pronotum darker, former almost black with blue-green reflections. These specimens resemble examples from Lee Co., Virginia and from Harlan Co., Kentucky (Plate 17, fig. 5), in having elevated, expanded humeri. In agreement with this observation is the following statement made by Chaudoir in reference to *germari*: "épaules qui sont arrondies comme dans le *viduus*." The pronotal margins are wider than in the more northerly race heretofore identified as *germari* and the sides and base of the pronotum are more coarsely and densely punctured.

10. **Steniridia andrewsi mutabilis** Casey

Irichroa mutabilis Casey, 1920

Irichroa mutabilis longicollis Casey, 1920

Irichroa mutabilis modulata Casey, 1920

Steniridia andrewsi germari Chaudoir–Valentine, 1935

Type, paratypes: 5♂s, U. S. National Museum; T. N. Brown (?), collector.

Type locality: Uniontown, Fayette Co., Pennsylvania.

Type ♂: length 23.6 mm.; width 8.8 mm.; head index .43; pronotal index .97; elytral index .69; abdominal index .42; tarsal index .64. *Color:* above, head blue-black, pronotum and elytra violaceous with blue reflections (Plate 17, fig. 6).

Topotypes, chosen from a large series to illustrate the greatest range of variation within the series which includes two male paratypes of *mutabilis*, the male type and two female paratypes of *longicollis* and the unique male type of *modulata*: 7 ♂s: length 20.7–24.2 mm.; width 8.2–9.0 mm.; head index .43–.48; pronotal index .88–.97; elytral index .67–.72; abdominal index .40–.45; tarsal index .60–.71. 5 ♀s: length 24.2–27.2 mm.; width 9.2–10.7 mm.; head index .44–.46; pronotal index .90–1.02; elytral index .68–.70; abdominal index .43–.46. *Color*: above, violaceous with blue-green reflections, especially on head, elytra often with aeneous cast, sometimes almost black; below, black, epipleurae faintly purplish; tarsi rather dark brown.

The Uniontown colony of *andrewsi* differs from typical *germari* in averaging less convex and in having a narrower pronotum, less expanded humeri and, in the male, a more extensive anterior tarsal pad development. The sixth and tenth elytral costae are usually conspicuously vermiculate. These distinctions make it necessary, under the standards of the present analysis, to recognize one of the three Caseyan names applied to examples of this colony. By page priority, *mutabilis*, given by Casey as a specific name, becomes valid as a subspecific name while his subspecies *longicollis* and *modulata* remain in synonymy as mere variants within the norm.

Mutabilis and its subraces (hardly worthy of nomenclatorial differentiation) seem to be restricted to the Ohio valley and to the highland plateau drained by the tributaries of this river. Specimens from the Monongahela valley at Fairmont, Marion Co., West Virginia, forty miles south of the type locality, possess pronota which are even more elongate (index as high as 1.05) than in the type colony; examples from an area in the Ohio basin including Cincinnati, Ohio, and Frankfort, Franklin Co., Kentucky, are all quite similar to *mutabilis* though the male specimens have tarsi as in *germari* proper. A single female taken by the author at Gawley Mountain, Fayette Co., West Virginia, 2600 ft., closely resembles *mutabilis* except for the sculpture which, in its coarseness, appears to exhibit the influence of higher altitude, and the elytral margin which is wider.

11. **Steniridia andrewsi waldensia** Valentine, 1935

Cychrus germari Chaudoir–Liebeck,1899

Holotype, allotype: ♂, ♀, Philadelphia Academy of Sciences; H. A. Pilsbry, collector.

Type locality: Sawyer's Springs, Hamilton Co., Tennessee.

The two examples comprising the type series of this race are the largest *andrewsi* yet recorded. They were taken near the summit of the southern end of Walden Ridge at about 1500 ft. elevation where conditions of relative dryness prevail.

The male tarsal equipment and size combine to place *waldensia* in group *germari* in spite of the more expanded antero-lateral pronotal margin which character links it, perhaps, with *parvitarsalis*.

CONCLUSIONS

1. A geographic or ecological race (subspecies) within a genitalically constant species of *Steniridia* (such as *andrewsi*) contains the entire, local population of that species as distinguished from neighboring and related segregates on the basis of diagnostic, though overlapping characters of size, color and sculpture.

2. Races adapted to lowland conditions have followed the river systems over relatively great distances and therefore have much more extensive ranges than races of upland habitat (*andrewsi* s. str., *mutabilis*, etc.).

3. Those races which prefer higher altitudes are ecologically limited to the particular range in which they have evolved. For these, the mountain chains rather than the drainage systems are the vehicles of distribution. Consequently, the higher the altitude preferred the more limited the range of the race and the more complete the isolation factor (*nantahalae*, *darlingtoni*, etc.).

4. In races of *Steniridia andrewsi*, as in species of *Steniridia*, size, color and sculpture may be correlated with the respective altitudes at which they are found; mean temperature and especially moisture conditions are indicated as factors influencing these characteristics.

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Errata in the above paper:

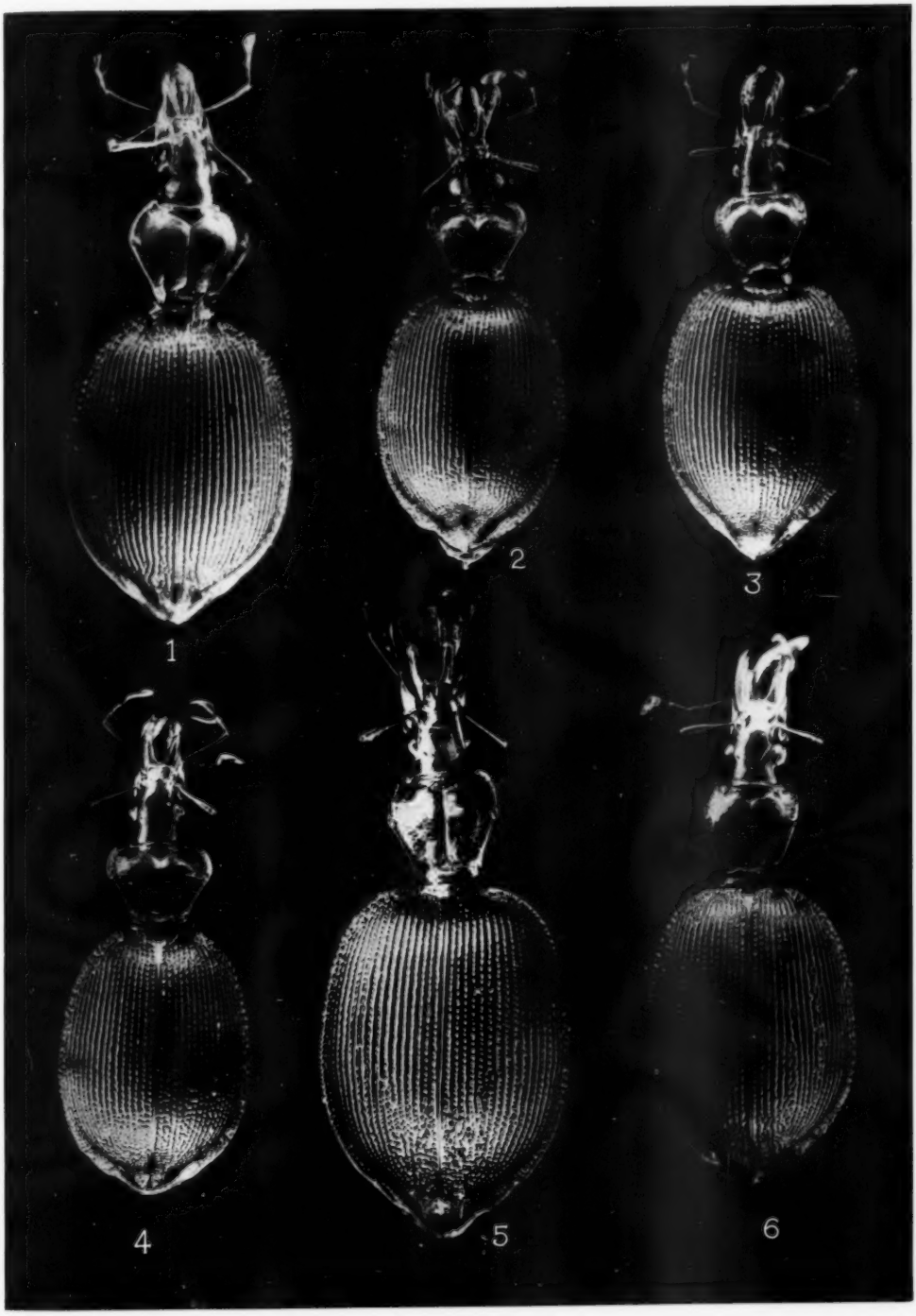
Page 351: second line from bottom, for Dowell read McDowell.
Page 354: sixteenth line from top, for "*mutabilis*" read "*modulata.*"
Page 358: third line from bottom, omit *andrewsi*.

EXPLANATION OF PLATE 17

(4 ×)

Fig. 1. *Scaphinotus Steniridia reflexa* Casey = *andrewsi amplicollis* Casey. Type ♀. Black Mountains, North Carolina. W. Beutenmuller, 1912.
Fig. 2. *Scaphinotus Steniridia andrewsi nantahalae* n. ssp. Type ♂. Cashier's, Jackson Co., North Carolina. J. M. Valentine, 1931.
Fig. 3. *Scaphinotus Steniridia andrewsi saludae* n. ssp. Type ♀. Melrose, Polk Co., North Carolina. W. F. Fiske, 1903.
Fig. 4. *Scaphinotus Steniridia andrewsi barksdalei* n. ssp. Type ♂. Mt. Guyot, Swain and Haywood Cos., North Carolina. L. Barksdale, 1934.
Fig. 5. *Scaphinotus Steniridia andrewsi germari* Chaudoir. ♀. Pine Mountain, Harlan Co., Kentucky. W. Stone, 1921.
Fig. 6. *Scaphinotus Steniridia andrewsi mutabilis* Casey. Type ♂. Uniontown, Fayette Co., Pennsylvania. T. N. Brown (?).

PLATE 17



NOTES ON THE HISTORY OF THE GERM CELLS IN THE TOADFISH (OPSANUS TAU)*

By Eugene P. Odum

Plates 18 and 19

It seems to be definitely shown that germ cells are segregated at an early stage of development in many species of vertebrates and invertebrates. In the vertebrates so-called primordial germ cells are usually described as being first distinguishable, by their large size and rounded shape, in the peripheral endoderm of the embryo at a time when the germ layers have recently formed and are found to migrate through the developing tissues to a place under the dorsal peritoneum ventral to the Wolffian ducts, in which position the gonads develop. Concerning the subsequent history of these cells, especially as to whether or not they become the definitive germ cells, there is, however, a great diversity of observations. Among recent contributions to this question in the case of fish, Okkelberg (1921: brook lamprey), Hann (1927: *Cottus bairdii*), Goodrich *et al.* (1934: *Lebistes reticulatus*) have presented evidence that the primordial germ cells do form definitive germ cells. Hann's work is especially interesting because of its completeness. After a study of adult seasonal stages as well as embryos he concluded that the primordial germ cells were the source of all the definitive germ cells throughout life. In constrast Essenberg (1923) found that the primordial germ cells in Xiphophorus mostly degenerated at the time of sex differentiation, to be replaced by germ cells arising from the peritoneal epithelium of the ovarian cavity and sperm duct, respectively. Wolf (1931: *Platypoecilus maculatus*) and Butcher (1929: lake lamprey) found that primordial germ cells, although not undergoing degeneration, were supplemented with germ cells of peritoneal origin. Foley (1927) studying adult testis only of *Umbra limi* found abundant transition stages be-

* The material for this research was collected and reared in the Beaufort (N. C.) Laboratory of the U. S. Bureau of Fisheries and the author wishes to express his indebtedness to the Commissioner of Fisheries, Hon. Frank T. Bell, and the Director of the Laboratory, Dr. Herbert F. Prytherch, for the facilities there provided. Also to Professor H. V. Wilson for guidance and criticism during the course of the work.

tween stroma cells and germ cells. Similar lack of uniformity is found in the literature of other vertebrate groups.

Sink (1912) in a short paper published the results of a study of the germ cells of early embryos of the toadfish (*Opsanus tau*) up to 8 mm. in length. He was unable to find primordial germ cells earlier than in a 3.5 mm. stage at which time they were located in the endoderm of the tubular gut. In slightly larger specimens they were located in the mesentery and under the Wolffian duct. In the 8 mm. stage a gonad had formed as a small sac containing the germ cells.

Since the toadfish gave promise of being a favorable form, a study was undertaken of later embryos and larvae in an effort to determine, if possible, the fate of the early segregated germ cells and the origin of the definitive cells.

The following observations are based on a study of a series of embryos from 6 mm. body length to larvae of about 23 mm. length. The series covers the development of the gonad through the period of sex differentiation.

Embryos 6 mm. in length. The body of the embryo is well formed although the yolk sac is still relatively enormous; the embryo is perched upon a large egg-shaped yolk sac. The germ cells are conspicuous at this stage (figs. 1a, 2). They lie under the dorsal peritoneum just ventral to the Wolffian ducts. The distribution of the germ cells is remarkably uniform; they lie on each side in a practically continuous stripe one cell thick and from one to three cells wide. A longitudinal section of this stage (fig. 1a) shows that the germ cells are closely appressed one behind the other. A gonad, as an organ projecting into the coelom, has not yet formed.

The germ cells occur over a length of 0.75 mm. in the posterior region of the embryo, extending anteriorly to the region of the liver and posteriorly almost to the end of the coelomic cavity. There are approximately 250 germ cells in the particular embryo studied.

Close examination of these cells reveals that they possess the typical characteristics of primordial germ cells as described for the vertebrates. They are large and rounded in shape, rivaled in size only by the differently shaped cells of the gut epithelium. They stain lightly as compared with the mesenchyme cells. The nucleus is spherical and contains a large darkly staining nucleolus, sometimes two, and numerous chromatin granules. Large chromatin granules are often aggregated just inside the nuclear membrane, smaller ones are scattered through the central portion of the nucleoplasm; a reticular network is present.

The cytoplasm stains lightly with eosin. The yolk granules possessed by primordial germ cells at segregation have apparently been absorbed. Material was not at hand for examination of earlier stages, but in view of Sink's work on the early stages and the general occurrence of similar cells in the early stages of many fish, there is no reason for supposing that these cells in the 6 mm. embryos are anything but primordial germ cells which have originated in the endoderm and migrated to the place they now occupy. We wish now to see, if possible, what part they play in the development of the sex glands.

Embryos 7 mm. in length. Although the stage is but slightly larger than the last there has been considerable activity in the genital region. In the middle part of this region, a conspicuous gonad has formed consisting of a peritoneal sac containing germ cells (fig. 3, just below the Wolffian duct). In the anterior and posterior portions of the genital stripe or ridge gonad formation has not progressed so far, and from a study of these regions we get an idea of the precise manner in which the gonadial fold forms. The cells of the peritoneal membrane on either side of the germ cells apparently divide rapidly, pushing the germ cells and their peritoneal covering out into the coelom. There is no evidence which indicates that any of the mesenchyme cells lying dorsal to the germ cells enter the gonad; the two layers forming the neck or "mesentery" of the gonad are from the beginning appressed closely. The gonad is then formed only from peritoneal cells and germ cells, the former active, the latter passive. The stroma cells which later occupy the interior of the gonad along with the germ cells originate from the peritoneal covering.

It has been suggested that the germ cells perhaps are not passive during the formation of the gonad, but exert some sort of activating influence on the peritoneal cells. Such might conceivably be the case, but the immediate presence of germ cells is not necessary for the formation of a gonadial fold in the toadfish. Anterior and posterior to the germ cell region the gonadial fold forms although there are no germ cells in the fold or in the neighborhood.

Embryos 9 mm. in length. The gonad now appears in section as a club-shaped organ (fig. 4) somewhat deeper dorsi-ventrally than in the 7 mm. embryo. The germ cells, to all appearances, remain unchanged, although their number in the particular embryo studied is only about 171. They show no indication of degeneration or division. The stroma cells of the gonad, however, have increased and now make up a large part of the interior of the gland. The germ cells are in consequence no

longer closely appressed but are surrounded by stroma cells. In front of this region containing germ cells, the gonad for a distance of about 450 μ, though well formed, contains no germ cells. Also for about 170 μ behind the germ cell region, a gonadial fold has formed with no germ cells.

Embryos 11 mm. in length. Sections of this stage (fig. 5) show a similar condition of the gonads. The stroma cells have, however, increased somewhat; dividing stroma cells are seen. The gonadial mesentery has become thinner. The germ cells are somewhat smaller, certainly, in some cases; the nucleus is still of the same character, but the cytoplasm is more limited, as if the pressure of the stroma has caused a decrease in the size of the cell.

The length of the gonadial fold is now about 2 mm., and the germ cells number about the same as in the preceding stages (actual count 220). The anterior 500 μ and the posterior 210 μ of the gonads contain no germ cells. In the middle region the germ cells are now quite scattered in the more abundant stroma. A longitudinal section of the gonad shows that the germ cells are no longer closely appressed, nor quite uniformly distributed. Stroma intervenes between them.

Embryos 15 mm. in length. Sex differentiation has taken place in this stage. In the males the extreme posterior portion, and this portion only, of the gonadial fold enlarges, and a slit, the sperm duct, shows distinctly in the stroma (fig. 6).

In the case of the females, the gonad along the whole length of the gonadial fold was found to have increased in size without an especially great enlargement in the posterior region. A groove or fold, the first indication of the formation of the ovarian cavity, is to be found on the external side of the anterior part of the gland (fig. 7). Also changes in the histological appearance of the interior indicate that active reorganization of the gonad is in progress.

Embryos 15 mm. in length, male. The remarkable feature in the male gonad is that the portion which enlarges and differentiates (fig. 6) is so short (about 90 μ in length), while the rest of the gonad, instead of becoming larger, is, if anything, smaller than before. There are no germ cells in the enlarged portion, nor were there any in this general region during earlier stages. Furthermore, the germ cells in the small part of the gonad in which they are present are much smaller than before, roughly about half as large as those in the 6 mm. stage. The cytoplasm of the cell is quite restricted as compared with the earlier condition. The number of germ cells is also smaller. One embryo contained about

98 cells, another 106. The most anterior region as before contains no germ cells.

Embryos 15 mm. in length, female. Sections of the female embryo make it evident that extensive reorganization is in progress over the whole length of the gonadial fold (figs. 7, 1d). It appears that a change is taking place both in the primordial germ cells and the stroma of the gland. In the first place the germ cells are fewer and smaller, the diminution in size being due to a decrease in the cytoplasm (as is the case with the male of this stage and to some extent in the 11 mm. embryos). Furthermore, many of the cells have an abnormal appearance. In a great many cases the cytoplasmic body is irregular and separated by a considerable space from an outer wall made up of stroma cells (figs. 1b, 1c, 7). In a cell or two the nucleus appears to have divided into irregular portions and there is no trace of a nucleolus. In most cases the nucleus is found to have become smaller and more deeply stainable. The changes would seem to be degeneration changes, indicating final disappearance of the primordial germ cells.

The stroma itself seems to be undergong extensive reorganization. Formerly (fig. 5) it presented the appearance of a tissue in which the nuclei were closely crowded and the limits of the cytoplasmic bodies not definitely observable. Now the stroma in one and the same gonad presents quite different appearances in different regions. In the more posterior region (fig. 7) the dense tissue of the earlier stage has been transformed into loose mesenchymatous tissue with plenty of watery space between the cytoplasmic areas. In the more anterior region (fig. 1d) the nuclei are spaced distinctly further apart than in the earlier stage and the internuclear substance is certainly very largely cytoplasmic. This change is of great interest in view of the pictures presented by the gonads in older stages (figs. 10, 11, 12). It strongly suggests that stroma cells have begun to transform into germ cells.

The count of the primordial germ cells in one embryo is about 116, fewer than formerly. Also, the cells have become still more scattered, so much so that several consecutive sections often show no germ cells. A large blood vessel has formed in the upper center of the gonad.

Embryos 18 mm. in length, male. The enlargement of the extreme posterior portion of the gonadial fold to form a testis which was observable in the 15 mm. embryo, male, has continued. Moreover, the right and left testes have fused so that we now have a single very large gonad lying under the immense urinary bladder (fig. 8, u. b.) The sperm ducts of the originally paired testes are to be seen near the center of the gland.

Also, secondary ducts leading in general towards the primary ducts have formed by dehiscence in the stroma. At the periphery, the cells have begun to aggregate to form masses suggestive of the cysts or tubules of the adult testis. The remarkable fact, as before, is the absence of germ cells in the enlarged portion, which is still short (about 220 μ long) as compared with the total length of the fold, and contains only a mass of stroma cells.

As before, the much longer anterior portions of the gonadial folds, containing the primordial germ cells, are quite small in transverse section (fig. 9). The part immediately anterior to the enlarged portion is the smallest of all. The gut is here enormous, and the gonad is greatly flattened.

The germ cells themselves (fig. 9) have the same general appearance as in male embryos of the preceding stage. They are small and with a darkly staining nucleus. They are, however, sometimes found in nests (fig. 9) indicating that some division is taking place, whereas formerly stroma intervened between the cells. A total of 312 germ cells was counted in one embryo indicating an increase. A small blood vessel occurs at the base of the mesentery.

The extreme anterior portion of the gonadial fold in the region of the liver contains no germ cells and is apparently being absorbed. It is very small and the mesentery very thin. The total antero-posterior length of the gonadial fold is about 2.8 mm.

Embryos 18 mm. in length, female. The difference between male and female gonads at this stage is profound. The whole middle and anterior portions of the gonad, instead of being quite small, have enlarged two or three times to form an ovary of good size (figs. 10, 11, 12). Furthermore, the ovarian cavity is now conspicuous. In the more posterior portion the formation of the cavity has just begun (fig. 10). Two folds appear on the external surface, one, which had already appeared in the 15 mm. stage, at the base of the mesentery, the other projecting over the lower surface of the ovary. Progressing anteriorly the ovary becomes somewhat wider and the dorsal fold is found to have grown down and the ventral fold up (fig. 11). In the anterior region (fig. 12), the two folds have met and fused, thus forming an ovarian cavity crescentic in transverse sections.

Whatever be the exact nature of the extensive reorganization which was described for the preceding stage, the result is striking. We now have a long gonad in which the germ cells number over 2,000 and are everywhere abundant. They form nests in the ovary of 5 to 10 or

more cells, three or four nests appearing in a section (fig. 11). They thus occupy practically the whole interior of the gonad. The stroma is restricted to the periphery of the organ, a few cells which mark the boundaries of the germ cell nests, and cells that have aggregated around the blood vessel which has shifted to the mesial side.

The cell boundaries of the individual germ cells are in most cases distinct, although sometimes difficult to make out. Otherwise the cells resemble the typical primordial germ cells though smaller. The chromatin granules in the nucleus are also smaller and more evenly distributed.

Posteriorly, the ovaries are continuous with a median mass of stroma concerned with the formation of the oviduct.

Embryos 21 mm. in length, male. They are much the same as the 18 mm. embryos but show certain advances. The gonadial fold is divided into three regions as before: The enlarged posterior part fused with its fellow which has not increased in length (228 μ), the long middle part (2.4 mm.) containing the whole crop of primordial germ cells, and the anterior, now rudimentary part. The enlarged and differentiated part contains virtually no germ cells. Examination of the developing tubules at the periphery, however, gives some evidence which may indicate a beginning transformation of stroma cells into germ cells. At any rate stroma cells are frequently observed to be aggregated around a cell, the nucleus of which is spherical, showing a nucleolus. The long middle portion of the fold containing the germ cells remains as before relatively very small in transverse section. Where the gut is large the gonads have in some places fused with the mesodermal layers of the intestine, appearing as small projections on the wall of the latter. In the more anterior part the gonad, though small, is lobulated, that is, it bulges out at several points. As was recorded for the 18 mm. male embryos, there is some evidence here of division of the germ cells in that one or two mitotic figures were found. The number of germ cells in a typical case is 226.

Embryos 21 mm. in length, female. Conditions in this stage are quite similar to the preceding. The ovary has become slightly larger. The folding process has proceeded posteriorly until only a small part of the ovarian cavity remains open to the coelom. The germ cells, as before, are packed in the interior of the gland. Some of the germ cells, however, now have vacuoles in them, and sometimes the nucleus is irregular, indicating more degeneration. The oviduct now opens into the cloaca just in front of the opening of the urinary bladder.

Embryos 23 mm. in length, female. The ovary (fig. 13) is now in transverse section much larger than in the preceding stage, and contains, instead of germ cells of uniform small size, germ cells in all stages from small ones to enormous ones that are clearly young oocytes. The small cells occur often in groups. The large ones have a large nucleus containing a large excentric nucleolus and fine chromatin granules. The cytoplasm of the cell stains well with haematoxylin, probably due to the formation of yolk. The intermediate cells of all sizes show that the young ova arise from the small germ cells.

DISCUSSION OF OBSERVATIONS

The stages from 15 mm. to 23 mm. covering the period of sex differentiation are the interesting ones in this study. There is little change in the primordial germ cells up to the time of sex differentiation. As we have seen, in the male, only the posterior part of the gonadial fold enlarges to form the anlage of the testis. This part contains no germ cells. It seems reasonable to conclude, therefore, that if this portion becomes the functional testis or part of it, germ cells must arise from stroma cells. The anterior part of the fold containing all the germ cells behaves in a puzzling way. While the posterior part is becoming greatly enlarged and undergoes considerable differentiation, the anterior part remains small and shows no sex characters (compare figs. 8 and 9). The germ cells in it are small and scattered, although some division is apparently taking place. Whether this part later enlarges to form part of the testis or whether it degenerates could only be determined by study of later stages.

In the female the situation is also puzzling. In the 15 mm. embryos it is evident that a change is taking place; the primordial germ cells are few, scattered, and some are sickly looking. In the next stage (18 mm.) the ovary contains over 20 times as many germ cells. Comparison of fig. 1d and figs. 10–12 is suggestive as to the manner in which these numerous cells might have arisen, that is, from groups of stroma cells. Although intermediate stages would be necessary to settle the point, it seems highly improbable that all of the 2,000 or more germ cells arose from the few scattered primordial cells of the 15 mm. stage. In the latter stage there are mostly stroma cells within the gland with a relatively few germ cells; in the 18 mm. stage the situation is reversed: there are mostly germ cells in the interior of the ovary and relatively few stroma cells. That the numerous germ cells (or at least some of them) of the 18 mm. embryos, apparently a second crop arising from

stroma cells, are true germ cells comes out in comparing sections of this stage with sections (fig. 13) of the 23 mm. female in which all stages of transformation of small germ cells into large oocytes are seen.

Referring to the literature we find that these observations on the toadfish do not correspond with observations on other fish except in a very general way. Other investigators (Okkelberg, Essenberg, Hann, Wolf, and Goodrich *et al.*) found that regardless of their later history the primordial germ cells behaved in a characteristic manner at the beginning of sex differentiation. In the female the cells enlarged; in the male they divided without enlarging to form nests at the periphery of the gland. In the toadfish the primordial germ cells are small and scattered at sex differentiation. The only criterion for sex differentiation is the behavior of the gland itself. No author describes a wholesale transformation of germ cells in the interior of the young ovary as my observations seem to indicate is the case with the female toadfish although Essenberg and Wolf describe the proliferation of germ cells from the epithelium of the ovarian cavity, and Foley the transformation of stroma cells in the adult testis. The entire absence of primordial germ cells in the developing testis (or part thereof) is unparalleled in any of these accounts. Finally, the observations of the toadfish did not bring out anything to indicate a condition of juvenile hemaphrodism as described by Okkelberg in the brook lamprey, or a reversion of sex as Essenberg believed takes place in some cases in Xiphophorus.

SUMMARY

(1). The primordial germ cells immediately before the formation of the gonad are evenly distributed along a considerable part of the posterior coelomic region.

(2). The gonadial fold is formed entirely from the peritoneum and includes the primordial germ cells.

(3). The gonadial fold extends anteriorly and posteriorly beyond the region of the germ cells.

(4). As the gonad develops, the primordial germ cells become scattered as a result of the increase in the stroma and the increase in length of the gonadial region. The count of germ cells, allowing for individual variation, is the same for all the stages of the indifferent gonad.

(5). The male sex is indicated by the enlargement of the posterior portion of the gonadial fold, and by the appearance of a sperm duct as a slit in the interior of the gonad.

(6). The female sex is indicated by the appearance of a groove or fold

on the external face of the gonad at the base of gonadial mesentery, and later by the formation of a second fold at the free edge of the gonad. The stroma also undergoes changes. Groups of spherical stroma nuclei surrounded by considerable cytoplasm are seen.

(7). The primordial germ cells of both sexes at sex differentiation are small and greatly scattered. Degeneration of cytoplasm was noted in germ cells of the female. In the male no germ cells are found in the enlarged part of the gonad.

(8). Germ cells in the female increase from about 100 at the 15 mm. stage to over 2,000 at the 18 mm. stage. Although the conclusion is not to be considered final, the evidence indicates that the latter germ cells are a second crop derived from groups of stroma cells. Some of the cells enlarge later to form oocytes; some also show evidence of degeneration.

(9). In the male, the posterior portion of the gonadial fold which contains no germ cells continues to enlarge and differentiate, while the anterior part containing small primordial germ cells remains small and indifferent in character.

UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

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EXPLANATION OF PLATES

(Photomicrographs except fig. 1)

PLATE 18

Fig. 1. Camera lucida drawings. Fig. 1a, part of a longitudinal section of 6 mm. embryo showing the uniform arrangement of germ cells before formation of the gonad. Figs. 1b, 1c, oil immersion drawings of primordial germ cells from 15 mm. embryo, female, showing retracted condition of cytoplasm. Fig. 1d, section of gonad of 15 mm. embryo, female, showing spherical

stroma nuclei surrounded by considerable cytoplasm; no primordial germ cells in this section.

Fig. 2. 6 mm. embryo. Part of transverse section through the trunk region showing large primordial germ cells, three on each side, under the peritoneum ventral to the Wolffian ducts. ×143.

Fig. 3. 7 mm. embryo. Part of transverse section showing the gonad as a fold of the peritoneum including four primordial germ cells. The Wolffian duct lies dorsal, and a large intestinal vessel ventral to it. ×143.

Fig. 4. 9 mm. embryo. Transverse section. The gonad is now club-shaped in cross section and contains stroma cells as well as primordial germ cells. ×143.

Fig. 5. 11 mm. embryo. Transverse section. The stroma has become more abundant within the gonad; the gonadial mesentery has become thin. The space between the mesodermal covering and the columnar epithelium of the gut is an artifact. ×143.

Fig. 6. 15 mm. embryo, male. Transverse section of posterior part of gonadial fold. The gonad has enlarged and a sperm duct has appeared as a slit in the interior. A part of the urinary bladder (paired at this level) is seen in the corner at the right; the Wolffian duct is above and at the right. Midline of body is towards the left. ×143.

PLATE 19

Fig. 7. 15 mm. embryo, female. Transverse section of the posterior part of the gonadial fold showing two primordial germ cells and a large blood vessel in the center. The cytoplasm has shrunken in the lower cell. The stroma has become mesenchymatous. The commencing ovarian groove at the base of the mesentery to the right is just perceptible. ×143.

Fig. 8. 18 mm. embryo, male. From a transverse section. The greatly enlarged posterior part of the fused gonadial folds, forming the (double) testis, lies under the very large urinary bladder (u. b.). Primary and secondary sperm ducts are seen, and on the right side three tubules are forming. The testis is composed entirely of stroma. g = gut. ×33.

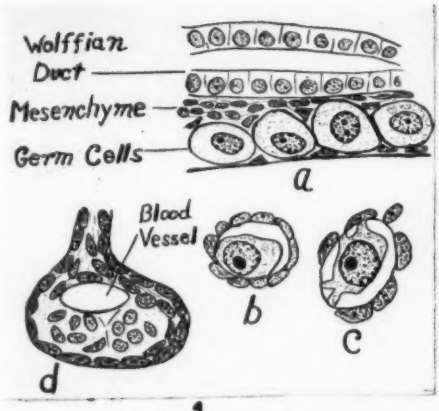
Fig. 9. 18 mm. embryo, male. Transverse section of anterior part of the gonadial fold. The gonad is very small and contains small groups of crowded primordial germ cells, and a small blood vessel. The Wolffian duct above it is cut three times. ×143.

Fig. 10. 18 mm. embryo, female. Transverse section of the ovary in the posterior region showing beginning formation of the ovarian cavity. The mid line of the body is towards the left. The space within the ovary is the lumen of a blood vessel. ×143.

Fig. 11. Same embryo. Section of the ovary anterior to last figure. Ovarian folds are approaching. ×143.

Fig. 12. Same embryo. Section through the anterior part of the ovary. The ovarian cavity is complete. Numerous germ cells are shown in all three figures (10–12). ×143.

Fig. 13. 23 mm. embryo, female. Transverse section of the ovary showing germ cells in all stages of enlargement to form oocytes. The lateral wall (left side) of the ovarian cavity is very thin and has collapsed. ×143.



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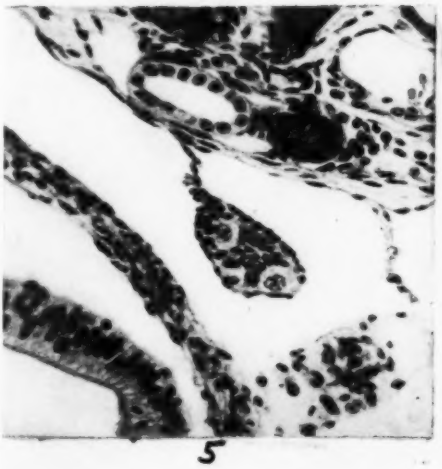

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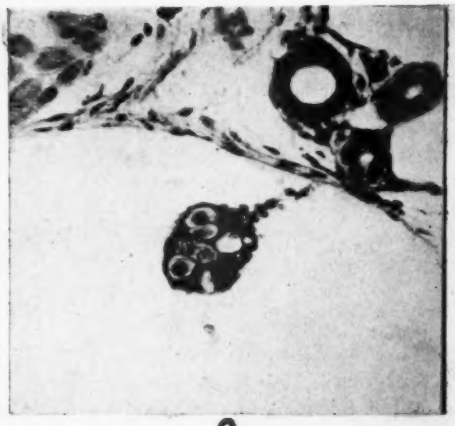

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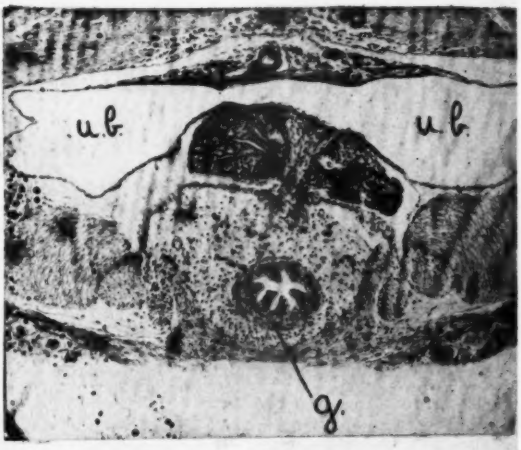

8

MORE PRIMITIVE MOSS-MITES OF NORTH CAROLINA

By Arthur Paul Jacot

Plate 20

The species of Oribatidae here described are all Hypochthoniinae as delimited in my earlier paper (2). The species of Brachychthonius are the smallest of the Oribatidae and colorless to orange. Although common in all litters they are usually overlooked because of their minute size and pale coloring. The species here treated fall into two groups—the smooth and the "sculptured." In the smooth species the bristles usually occupy the same relative positions. The specific differences are therefore limited to the modifications of the bristles themselves and of the pseudostigmatic organs. In the sculptured species the same remarks obtain except that the sculpturing forms an additional and a conspicuous specific character. Breadth of abdomen is a much more constant measurement than length.

KEY TO GENERA OF EAST AMERICAN HYPOCHTHONIINAE

1. Abdomen with only two distinct transverse plates..... *Hypochthonius*
1. Abdomen with three transverse plates.....2
2. Anterior transverse suture interrupted or indistinct in middle...*Eniochthonius*
2. Anterior transverse suture quite distinct throughout.....3
3. Size small (less than 0.2 mm. long); sides without lateral plates *Brachychthonius*
3. Size larger (0.25 mm. or more); sides with four small lateral plates *Eobrachychthonius*

Brachychthonius perpusillus (1, p. 220, fig. 41)

Figure 1

Diagnostic characters: Bristles of dorsal face of medium length, somewhat acicular-foliaceous, the sides flaring upward and laterad from the "midrib;" pseudostigmatic organs with short head bearing about five bristles longitudinally in several rows.

Description: Color pale; size medium (for the genus): length 0.17 mm., breadth 0.096 mm.; rostrum broad, rostral bristles inserted fairly close to anterior edge, projecting well beyond end of rostrum; interlamellar bristles inserted rather far forward, length of rostral bristles

distant from rostrals, broader than most of the bristles, deeply longitudinally folded (see figure to left of numeral 1); exopseudostigmatic bristles with projecting midrib (figure to right of numeral), the sclerotized ring large and poorly defined, no clear-cut dorsolateral (lamellar) ridge; interlamellar bristles erect, inserted quite close to end of pseudostigmata, with more than one longitudinal rib; notogastral bristles all of one pattern; their positions (as in figure 1) quite constant, a2 usually extending posteriad above edge of abdomen, a3 on ventrolateral face, directed laterad; bristles of transverse plate II inserted slightly nearer anterior edge of plate than to posterior edge, especially c2.

Cotypes: Twelve specimens from the F-layer of litter of isolated, forty-year-old short-leaf pine stand among closely grazed *Andropogon* pastures on Asheville-Brevard road twelve miles southwest of Asheville, N. Car.; taken October 15th, 1934, slide 34F10.2B1.

Brachychthonius latus *sp. nov.*

Differs from *B. perpusillus* in that all bristles of dorsal aspect are longer, more slender, and curved; interlamellar bristles directed forward; dorsal plate bristles of second and third transverse rows overlap insertion of the bristle behind them. Size and color seem to be the same though *B. latus* is slightly broader, especially at the shoulders. The bristles are inserted in exactly the same places.

The following are identical with specimens from Regensburg, Germany: sixty-eight specimens from upper part of F-layer of litter of thirty-year-old white pine plantation, Biltmore estate, eight miles from Asheville on Brevard road; taken October 8, 1934, slide 34F9-2h.

Brachychthonius fimbriatus *sp. nov.*

Figures 2 to 4

Similar to *B. perpusillus* but pseudostigmatic organs long, distal end curved backward and downward, head furnished with ten to twelve bristles linearly; exopseudostigmatic rings well developed, angular, the bristle appearing coarsely triquetrous to quinquetrous; lamellar and interlamellar bristles erect, and, like the notogastral bristles, expanded laterally as very thin wings which are more or less transversely wrinkled and fimbriate (figures 5 and 6), membrane narrowing rapidly to distal end. This membrane is so fine as to be very difficult to discern even under oil immersion, except where the transverse wrinkles are seen along their faces when enough density or shadow is formed to become more visible. The midrib of the bristle is always quite distinct, giving

the animal the appearance of *B. latus*. The fimbriation is more visible in dirty specimens, and on shoulder bristles and bristles of posterior edge of abdomen. Color usually pale. Size large (for the genus): length 0.197 mm., breadth 0.1 mm.

Cotypes: Thirty-seven specimens from sod of *Andropogon* bald (Glen Bald), Bent Creek Experimental Forest, ten miles southwest of Asheville; taken April 17th, 1935, slides 34F31-2, -3, and -6.

Brachychthonius bifurcatus *sp. nov.*

Figures 5 and 6

Differs from *B. perpusillus* in that all bristles of dorsal aspect are shorter, more slender, and stiff; rostrum distinctly set off; ridges on sides of cephaloprothorax better developed (figure 5); pseudostigmata with funnel straight and stout; pseudostigmatic organs with head more compact so that no distinct bristles are evident, distal end deeply notched (figures 5 and 6); bristles of middle segment inserted *posterior* to transverse center of plate; color quite a distinct orange-yellow, the bristles colorless; size of females: length 0.184 mm., breadth 0.1 mm.

Cotypes: Fifteen specimens from litter of twelve-year-old *Andropogon* pasture with scattered two to three year old pines, Cook property, Brevard road nine miles from Asheville; taken February 19th, 1934, slide 34F26-1.

Brachychthonius italicus spiciger *comb. nov.*

Figure 7

The first sculptured Brachychthonius to be described is *B. brevis italicus* (1, p. 220). As *B. italicus* is not a subspecies of *B. brevis* Michael (non Berlese = *B. berlesei* Willmann (7, p. 160) it stands as a full species. Thus *B. brevis expolitus* (1, p. 220) is a short bristled smooth species (or subspecies) closely related to *B. brevis* Michael (4). *Brachychthonius brevis spiciger* of Lake City, Florida is described (1, p. 220) as having a longer, slenderly fusiform pseudostigmatic organ and somewhat different sculpturing than *B. berlesei* which I recognize as a distinct species. The common sculptured species of the southern Appalachians which I regard as *B. spiciger* resembles much more closely *B. italicus* in sculpturing. I am therefore calling it *B. italicus spiciger*.

Diagnostic characters: Bristles of dorsal aspect quite short and fine; pseudostigmatic organ head long, slender, inconspicuously roughened (spiciger = bearer of wheat heads); sculpturing of angular designs (figure 7); posterior edge of abdomen broadly rounded, not angled.

Description: Color pale; size medium: length 0.17 mm., breadth 0.09 mm.; cephaloprothorax somewhat angular, with well developed ridges; rostrum distinct, the bristles prominent, longer than the other body bristles; lamellar bristles plicate, exopseudostigmatic ring conspicuous, angled; pseudostigmata with slender cells; vertex with a cluster of three pairs of more or less rectangular areas formed by slender ridges; bristles a2, a3, and b3 quite close to each other, bristles c1 and c2 nearer posterior than anterior edge of plate.

Material described: Forty-seven specimens from L-layer of litter of dogwood of thirty-year-old-field woodland, Bent Creek Experimental Forest; taken September 20th, 1934, slide 34F4.1-2.

Brachychthonius rostratus *sp. nov.*

Figure 8

Diagnostic characters: Bristles of dorsal aspect very short, fine; pseudostigmatic organ head slender with four or five bristles in linear series; sculpturing comprised of clusters of ovate spots of granules (much resembling oil globules on the surface of bouillon) (figure 8, where the granules are indicated in a few of the spots); posterior end of abdomen deeply and broadly emarginate.

Description: Color pale honey yellow; size smallish: length 0.168 mm., breadth 0.0877 mm.; cephaloprothorax, especially anterior end, quite angular; rostrum distinct, bristles not discernible; ridges well developed, a secondary ridge mesad of the lateral; vertex with a row of four rectangular areas formed by slender ridges; bristles a2, a3, and b3 not discernible; bristles b2 inserted quite laterad; bristles c1 and c2 nearer anterior than posterior edge of plate.

Cotypes: Eight specimens from sod of *Andropogon* bald, top of Shut-in-Ridge, Bent Creek Experimental Forest; taken May 8th, 1935, slide 34F34-19.

Brachychthonius brevis Michael (4) is a smooth species with slightly serrate (see figure) rostral bristles, interlamellar bristles somewhat spine-like, abdomen almost parallel-sided, bristles of dorsal plates thick. If it were not for the serrulate rostral bristles, I would regard *B. perpusillus* as possibly synonymous. *Brachychthonius perpusillus* Berlese (1, p. 220) is very similar as to caliber and length of dorsal bristles, and length of pseudostigmatic organs. *Brachychthonius brevis* Willmann (7) has shorter and finer bristles and in that respect approaches *B. bifurcatus*. *Brachychthonius perpusillus* Willmann (7) has bristles

of dorsal face of abdomen resembling those of *B. latus*. All these European species are so poorly described and figured (except Michael's) as to make it impossible to make detailed comparisons. Henceforth special attention should be paid to character (relative length, breadth, and shape) of bristles, especially the rostral, exopseudostigmatic, interlamellar, and dorsal plate, as well as pseudostigmatic organs. The exact location of bristles of the middle transverse plate is important. Bristles b2 tend to migrate toward lateral edge of abdomen.

Eniochthonius pallidulus (3)

Specimens from the eastern states south to the southern Appalachians are identical with specimens from Regensburg, Germany. I have them from cove litter of the Bent Creek Experimental Forest.

Hypochthonius gracilis *sp. nov.*

Figure 9

Diagnostic characters: Body slender; interlamellar bristles erect, clavate; notogastral bristles twenty-four, long, overlapping, tapering, slightly curved.

Description: Color pale; size rather small: length 0.31 mm., breadth 0.14 mm.; rostrum fairly broad, edge smooth; rostral bristles rather short, not extending much beyond rostrum; lamellar bristles medium long, inserted in longitudinal middle of cephaloprothorax, directed backward; sides of cephaloprothorax with a short ridge; exopseudostigmatic bristles rather short; pseudostigmata with four cells, the organ with about ten cilia; no depressed angle at anterolateral corners of notogaster, nor at posterolateral areas; ventral plate with two rather short bristles each side; anal covers with two insertions; paranal plates with three insertions; genital covers transversely divided, each half with three mesal and two lateral insertions (figure 9); parasterna with bristles as in figure 9.

Cotypes: Twelve specimens from fallen, damp leaves, horticultural grounds, Gainesville, Fla.; taken February 29th, 1928, by Edgar F. Grossman, slides G30H1 to -H3.

This species also occurs in the Bent Creek Experimental Forest area, especially in litter of old woodlands. Five specimens from leaf litter, Rocky Cove, Bent Creek Experimental Forest; taken September 8th, 1934, slides 34F1-1 and -18. Four specimens from pine-oak woodland, section 18 of same Forest; taken October 1st, 1934, slide 34F7-3.

Hypochthonius rufulus carolinicus (2)

In this subspecies the pseudostigmatic organs have fifteen or more pectinations; the bristles of dorsal plate are closely barbed, long, curved, tapering. It is represented by small numbers in square foot samples of woodland litter.

Hypochthonius luteus (7, p. 24, pl. 16, figs. 16–19) (8, p. 21)

Specimens from the Bent Creek Experimental Forest area are identical with those from Regensburg in Bavaria. The pseudostigmatic organs have ten to thirteen pectinations; the bristles of dorsal plates are shorter than in *H. rufulus*, stiff, rough, and blunt.

Two specimens from sod of *Andropogon* bald, top of Shut-in-Ridge, Bent Creek Experimental Forest; taken May 8th, 1935, slide 34F34-20. One specimen from litter of old field grown to pines, Stradley Hill, nine miles southwest of Asheville on Brevard road; taken October 30th, 1934, slide 34F15.2o1.

U. S. FOREST SERVICE,
ASHEVILLE, N. C.

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PLATE 20

Brachychthonius perpusillus *sp. nov.*

Fig. 1. Dorsal aspect, legs omitted; ×529. Details of bristles free hand.

Brachychthonius fimbriatus *sp. nov.*

Fig. 2. Dorsal aspect of cephaloprothorax, and an additional pseudostigmatic organ; ×529.
Fig. 3. Posterior edge of abdomen with two of the bristles; ×529.
Fig. 4. A mesal bristle of abdomen; free hand.

Brachychthonius bifurcatus *sp. nov.*

Fig. 5. Dorsal aspect of cephaloprothorax, legs omitted; ×529.
Fig. 6. A pseudostigmatic organ; free hand.

Brachychthonius italicus spiciger (1)

Fig. 7. Dorsal aspect, legs omitted; ×529.

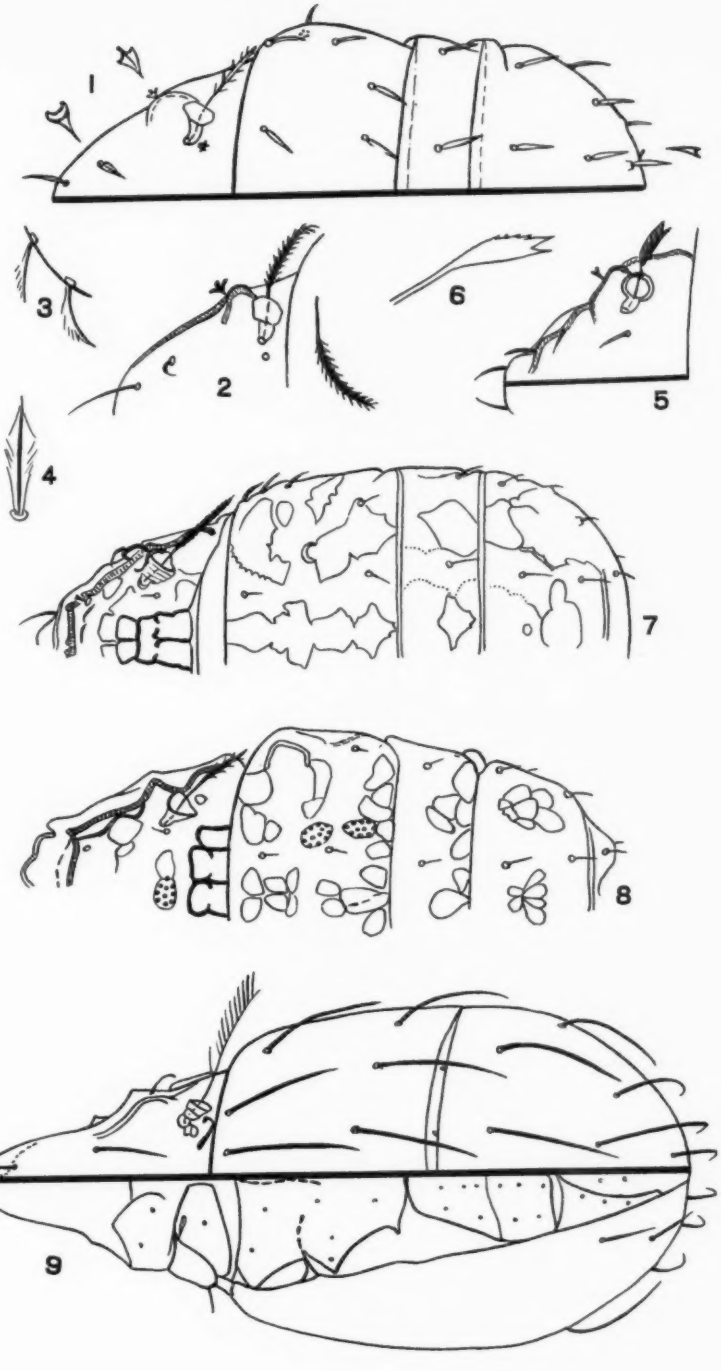
Brachychthonius rostratus *sp. nov.*

Fig. 8. Dorsal aspect, legs omitted; ×529.

Hypochthonius gracilis *sp. nov.*

Fig. 9. Dorso/ventral aspects, legs and mouth parts omitted; ×329.

PLATE 20



ORIBATA AND BELBA FROM FLORIDA (ACARINA, ORIBATINAE)

By J. W. Wilson

Plate 21

The material described in this paper was collected by Mr. Edward F. Grossman and a few others of the Experiment Station staff during the spring and early summer months of 1928. The collections were all made by collecting leaves and debris which were placed in Berlese funnels to trap the mites. The specimens were then mounted on slides in balsam. Jacot (1933 and 1935) gives a map of Florida showing the location of most of the points at which collections were made and the field notes of Grossman relating to these collections. This collection made by Grossman covers the whole state and represents a wide variety of ecological conditions. In this paper the number preceding the dash on each slide represents the lot number in Grossman's field notes. In the notes on places where collections were made, the year (1928) is not repeated and where no collector is named the specimens were collected by Grossman.

The author wishes to acknowledge Professor J. R. Watson's assistance in securing the collection and his continued interest in the study of this material. To Dr. Arthur P. Jacot the author is indebted for many helpful suggestions and much encouragement in the execution of this work. Dr. H. E. Ewing of the National Museum has very kindly loaned specimens for purposes of comparison.

In this paper the author has followed Jacot (1929 and 1934) in his definitions and restrictions of the tribe *Oribatinae* and the genera included therein. In these papers Jacot presents evidence indicating that *Oribata* Lat. belongs to the apterogastinae group of Acarina instead of to the pterogastinae where it was placed for a long time even by such careful workers as Michael. It is also shown that the genus *Damaeus* Michael is probably a synonym of *Oribata*.

Figures of *Oribata grossmani* sp. n. were sent to Professor Nathan Banks of the Museum of Comparative Zoology which he compared with his type of *O. angustipes* Banks. Professor Banks replied as follows: "Your figures do not represent *O. angustipes* but some allied species.

The long bristles on hind legs do not appear broken, and are alike on both hind legs, about as long as the long one near tip of tibia. However, there is a more important difference; the hind tarsi are much longer than your figure, the part beyond the swelling being fully twice as long as your figure. The tibia is fully as long as your figure. The front tarsus is also longer but not so much longer than your figure."

Oribata grossmani sp. n.

Figures 1, 5 and 8

DIAGNOSIS

Cephalothorax with longitudinal ridges each side joining a short curved transverse ridge in front of each pseudostigmatum (fig. 5), tectopedia I and II prominent. Dorsal part of body and legs thickly studded with short tubercles. Notogaster bearing on each side of anterior margin a stout forewaist spine which reaches almost to the base of the pseudostigmata. Legs very long and slender, bearing a few very long bristles.

DESCRIPTION

Rostrum rather long, tapering to a point and bearing above two pairs of long rostral hairs, one at sides, and one on the dorsum. Cephalothorax suddenly wider in front of coxae I, thickly studded with short tubercles, with ridges as shown in figure 5; exopseudostigmatic bristles short, fine, interlamellar bristles finely serrated, curved, extending back to the anterior margin of the notogaster; pseudostigmata funnel-shaped; pseudostigmatic organ very long, tapering to a fine thread-like end, finely serrated along basal portion (fig. 8); tectopedia I and II quite prominent, easily seen from both dorsal and ventral view.

Abdomen round, very convex with a wide lateral band between notogaster and ventral plate, notogaster not covering anterior lateral angles of abdomen, surface of notogaster covered with short tubercles, two rows of 8 curved, short, stout, finely serrated bristles near median line, three short, curved bristles near posterior margin. The anteriormost bristles are near the anterior margin and closer to the median line than the remaining bristles. On the anterior part of the notogaster there are two stout, curved spines (forewaist spines) reaching almost to the base of the pseudostigmata.

Ventral plate projecting as a shelf ventrad of leg II to leg IV, this shelf with well developed projections in front of leg III and in front of

and behind leg IV. Anal aperture near posterior edge of ventral plate, genital aperture close to anal aperture. Sides of anal covers almost straight, curving posteriorly, anterior edge deeply emarginate. Preanal and postanal bristles stout and short, close to the anal aperture. Preanal bristles located slightly ventrad of anterior margin of anal covers, lateral postanals at center of covers and the mesal postanal pair near posterior edge of anal cover; anal cover bristles short, nearer lateral edge of covers than mesal edge, the anterior near anterolateral angle, the posterior near center of covers and in line with lateral postanal bristle; paramesal bristles slightly anteriad of posterior angle of genital aperture. Genital aperture broadly oval; four genital cover bristles, the first two on middle line of cover, the posterior two nearer lateral edge, genito-thoracic suture not visible. Parasterna III and IV each bear three long fine bristles arranged nearly in a straight line, parasterna I and II each with a single bristle located as indicated in figure 5. Camerostome large, elongate oval, labial bristle inserted near the lateral margin.

Legs terminated by a single slightly curved hook, armed with short and long bristles. Leg IV the longest, leg II the shortest, legs very long and slender.

Leg I (fig. 1) longer than leg II. Tarsi fairly broad near proximal end, dorsal face with four bristles; proximal inserted at the broadest part of the segment, bristle 2 inserted far from 1 on slender distal part of tarsus, bristle 3 about one-third the distance from 2 as 2 is from 1, bristle 4 about equal distance between 3 and distal end of tarsus, bristles 2, 3, and 4 fine and short, only plainly visible under high magnification: ventral face with six bristles, bristle 1 more proximal than 1 of dorsal face, bristle 2 inserted at the point where the tarsus becomes narrower, bristles 3, 5, and 6 inserted opposite 2, 3, and 4 of dorsal face, bristle 4 equidistant between 3 and 5, bristles 3, 4, 5, and 6 similar to 2, 3, and 4 of the dorsal face, a lateral bristle inserted near dorsal bristle 1, borne on a tubercle, another inserted a little more distad and on the lateral median line, a mesal bristle inserted slightly distad of ventral bristle 1, another inserted on the median line and slightly more proximal of dorsal bristle 1, all bristles on tarsi smooth. Tibiae only a little shorter than tarsi, slightly swollen at distal end; two bristles on dorsal surface, a finely serrated major bristle midway between proximal and distal ends of tibiae, a smooth minor bristle inserted at the distal end; four smooth bristles on ventral surface spaced equidistant apart; two bristles on lateral surface; one finely serrated bristle inserted near ven-

tral bristle 3, one smooth bristle borne on a tubercle near dorsal surface and distal end of tibiae, no bristles on mesal surface. Genuals one-third length of tibiae, with two finely serrated bristles on dorsal face inserted very close together, one long, finely serrated bristle inserted on lateral face near ventral face and more proximad than the two dorsal bristles, one finely serrated, short bristle inserted on ventral face midway between proximal and distal ends, one long finely serrated bristle on mesal surface inserted on same transverse plane as lateral bristle but nearer dorsal surface (not shown in fig. 1). Femora long, slightly curved and enlarged at distal end; dorsal face with three finely serrated bristles, one longer and stouter than the other two, inserted near proximal end of segment, bristles 2 and 3 inserted near the widest part of segment; ventral face with two finely serrated bristles, bristle 1 inserted two-thirds distance from proximal end of segment, bristle 2 inserted near beginning of enlargement; a long finely serrated bristle inserted on lateral face on same transverse plane as bristle 2 on dorsal face, a finely serrated short bristle inserted on mesal face on the same plane as bristle 2 of the ventral face. Coxae small, cup-shaped, a single smooth bristle inserted at distal end.

Leg II (fig. 1) similar to leg I but each segment proportionally shorter. Tarsi with two additional bristles on dorsal face, no bristle rising from a tubercle on lateral face, two additional bristles on ventral face, two mesal bristles, lateral bristle finely serrated, all the other bristles smooth. Tibiae with one bristle on dorsal face, a long, stout finely serrated bristle on both lateral and mesal faces, these two bristles opposite, three bristles on ventral face. Genual with one bristle on each face, all finely serrated. Femora with two bristles on dorsal face, two on lateral face, one on ventral face and two on mesal face, all finely serrated. Coxae same size as coxae of leg I, with a long, curved, smooth bristle inserted on ventral face near distal end.

Leg III longer than leg II but not as long as leg IV. Tarsi shorter than tarsi IV, dorsal face with three bristles, bristle 1 inserted at widest part of segment, finely serrated, bristle 2 and 3 at distal end of tarsi, lateral face with one long, finely serrated bristle inserted slightly distad of dorsal bristle 1, ventral face with five short, smooth bristles; mesal face with two short smooth bristles, one inserted near proximal end and one slightly distad of the bristle on lateral face. Tibiae shorter and more robust than tibiae IV, one finely serrated bristle on dorsal face, two smooth bristles on lateral face, three on ventral face, one on mesal face. Genuals shorter than genuals IV, one long bristle on dorsal

face, one on lateral face, none on ventral face, and two on mesal face, all finely serrated. Femora stouter and shorter than femora IV; three short finely serrated bristles on dorsal face, one long, smooth bristle on lateral face, inserted at widest part of segment, no bristles on ventral face; one on mesal face, inserted on same transverse plane as bristle 2 of dorsal face. Coxae stouter than coxae IV and with two finely serrated bristles on dorsal face.

Leg IV longest and slenderest of all the legs. Tarsi with five smooth, short bristles on dorsal face, one long, finely serrated bristle on lateral face, four short, smooth bristles on ventral face, and no bristles on mesal face. Tibiae very long and slender, only slightly swollen at distal end, with one long, finely serrated bristle on dorsal face, one short finely serrated bristle on lateral face, no bristles on ventral face, five short, smooth bristles on mesal face. Genuals slender with one short finely serrated bristle on dorsal face; one very long (extending beyond distal end of tibiae), smooth bristle on lateral face, inserted near distal end; no bristles on ventral face; one short finely serrated bristle on mesal face. Femora long, slender, swollen at distal end, with three finely serrated bristles on dorsal face; a very long, smooth bristle on lateral face (not as long as bristle on genuals, but extending half length of tibiae); no bristles on ventral or mesal face. Coxae gourd-shaped, with a single short finely serrated bristle on dorsal face.

Color, reddish tan.

Dimensions of five specimens given in microns are as follows:

	Greatest	*Average*	*Smallest*
Total length of body	666	632	599
Length of notogastral plate	432	397	346
Breadth of notogastral plate	412	384	366
Interlamellar bristle span	80	77	66
Camerostome to genital aperture	166	146	133
Length of genital aperture	133	113	100
Breadth of genital aperture	133	116	100
Genital aperture to anal aperture	39	38	26
Length of anal aperture	120	94	66
Breadth of anal aperture	93	78	53

Specimens studied:

22 Specimens from Villa Tasso, Choctauhatchee Bay, Fla., from fallen leaves of oak, Magnolia, and hickory, May 18, R. W. Blacklock. Slide Nos. 101-D31, 101-D35, 101-D38, 101-D30, 101-D39, 101-D41, 101-D51. One specimen from *Asparagus plumosus* Pierson, May 18, Erdman West, Slide no. 100-D47. Two specimens from fallen water oak leaves, near

Mulberry, May 17, Erdman West, Slide Nos. 103-D48 and 103-D50. Four specimens from fallen leaves, Gainesville, April 20, Slides Nos. 73-D29, 73–D43, 73-D44, 73-D52. One specimen from dry leaves on ground, Bonita Springs, May 4, J. R. Watson, Slide No. 95-D37. Four specimens from fallen hickory leaves, Pinkoson Springs near Gainesville, March 14, Slide Nos. 33-D40, 33-D42, 33-D46. Seven specimens from fallen oak leaves, Devil's Mill-Hopper near Gainesville, April 4, Slide No. 75-D32. Two specimens from dry leaves, Gainesville, May 10, H. E. Bratley, Slide Nos. 90-D33, 90-D34. One specimen from fallen elder, Magnolia, oak leaves, Gainesville, February 29, Slide No. 29-D36. One specimen, leaves on beach, North Beach, St. Augustine, April 1, Slide no. 63-D45.

Holotype, Slide No. 101-D39.

Belba globifer florida subsp. n.

Figure 6

DIAGNOSIS

Differing from *B. globifer* in that the pseudostigmatic organ is smooth instead of slightly pectinate and that the spines projecting from the front of the abdomen are straight instead of curved.

DESCRIPTION

Cephalothorax broad and plain, without ridges, furrows or bands; rostrum rather short and rounded at anterior end, rostral bristles stout and curved inward; interlamellar bristles stout and curved outward; exopseudostigmatic bristle stout and curved forward.

Abdomen almost perfectly round; notogaster smooth, high, regularly arched; margin smooth; bristles long, stout, only slightly curved, there are eight bristles in each row placed near the center of each side of the notogaster; three short bristles near posterior margin, from the waist there are two stout straight, cone-shaped spines whose tip is some distance from the base of the interlamellar bristles.

Ventral plate well developed, round, not encroached on by the notogaster; triangular tooth projecting from margin between legs III and IV; anal aperture close to posterior margin, broadly rounded posteriorly, sides tapering slightly, anterior edges slightly rounded; anal covers convex with three bristles located as shown in figure 7; preanal bristle well toward lateral margin of ventral plate and on same transverse plane as anterior anal cover bristle; mesal postanal bristle near pos-

terior edge of anal aperture; two bristles posterior to transverse plane tangent to anterior margin of genital aperture, one near lateral margin and one near center of ventral plate; three bristles on transverse line anterior to genital aperture. Genital aperture almost round, rather large with five fine bristles on genital covers in positions indicated in figure 6; genito-thoracic suture not visible from ventral view. Sternum with two bristles (figure 6), parasternum II with one bristle.

Legs terminated by a single well developed hook, and armed with rather stout smooth bristles, legs IV the longest.

Legs I much longer than legs II, coxae without bristles. Femora with long slender proximal portion, large swollen portion distally, two stout bristles on dorsal face, no bristles on external side, two bristles on ventral face, one near center and proximal end of swollen portion, the other slightly distad of first and toward inner side, inner side with one bristle on same plane as the more proximal bristle on dorsal face. Genuals short, with two bristles on dorsal face, one on inner side, one near center and proximal end of swollen portion, and one on outer side. Tibiae two-thirds as broad as long, major bristle on outer side twice as long as the segment, a short fine bristle slightly distad on the dorsal face with a stout long bristle near center of dorsal face, a stout long bristle on same plane on the inner side, two bristles on about same plane on ventral face, one stout and long near center and one short and fine near inner side. Tarsi with short proximal neck, dorsal face with three bristles, outer side bearing short major bristle and two others on slender distal portion of segment, no bristles visible on ventral face, inner side with five bristles arranged as shown in figure 6.

Legs II with each segment relatively shorter than in leg I, longer than leg III. Coxae similar to those of legs I. Femora with long slender proximal portion and large swollen distal portion, two bristles on dorsal face, one on outer side, two on ventral face, one at proximal end of slender portion, and one near center of proximal end of swollen portion, one on inner side (fig. 6). Genuals slightly less than half as broad as long, one bristle on dorsal, outer and inner faces, none on ventral face. Tibiae with two bristles on dorsal face and one on outer side. Tarsi with five hairs on each of the inner and outer sides, three shorter and finer hairs on dorsal face, and three on ventral face.

Legs III shorter than legs II. Coxae gourd-shaped with a sharp tooth-like projection on the internal face, internal part of coxae rounded except for the tooth-like projection, external part neck-like, irregularly swollen distal portion, one short fine bristle on dorsal face, and one long

stout bristle on outer side. Femora with two stout, long bristles on dorsal face of swollen portion, one stout bristle on outer side. Genuals short and small with two bristles on dorsal face, one on outer side and one on inner side. Tibiae nearly half as long, one bristle on dorsal face, one on outer side, two on ventral face. Tarsi with five bristles on outer side, one on ventral side near distal end, four on inner side, none on dorsal face.

Legs IV with genuals reaching to end of abdomen. Coxae with inner rounded portion, long curved slender portion swelling into rounded squash-shaped distal portion with one stout bristle on ventral face. Femora half as broad as long with two bristles on dorsal face and two on ventral face. Genuals short, slightly enlarged at distal end, one bristle on dorsal, outer and ventral sides. Tibiae longer, not quite half as broad as long, one bristle on dorsal face, one on outer side and two on ventral face.

Dimensions of five specimens measured in microns are as follows:

	Greatest	*Average*	*Smallest*
Total length of body	413	391	366
Length notogastral plate	279	241	213
Breadth notogastral plate	246	207	186
Interlamellar bristle span	86	76	60
Camerostome to genital aperture	100	85	80
Length of genital aperture	73	70	60
Breadth of genital aperture	66	64	60
Genital aperture to anal aperture	33	23	20
Length of anal aperture	86	74	66
Breadth of anal aperture	66	64	53

Specimens studied:

One specimen from leaves of sweetgum, near Micanopy, April 17, Slide no. 74-D24. Cotypes: One specimen from hickory leaves, Pinkoson Springs, March 4, Slide number 33-D22. Two specimens, Bay Shore debris, Vero Beach, Erdman West, April 6, Slides nos. 67-D23 and 67-D19. One specimen, dry oak leaves, Horticulture grounds, Gainesville, H. E. Bratley, April 27, Slide no. 84-D27. One specimen, dry leaves, Gainesville, April 2, Slide no. 75-D6. One specimen, elder, Magnolia, and oak leaves, Horticulture grounds, Gainesville, February 29, Slide no. 29-D21; two specimens from pine and oak leaves, Wellborn, May 30, Slide nos. 115-D1 and 115-D11.

Belba michaeli (Ewing) 1909, p. 129

Under date of February 1, 1936, Dr. H. E. Ewing wrote in reply to a request for the loan of a type specimen of *Damaeus michaeli* that "All

of the original types of this species have been lost or misplaced, but I have an autotype specimen which was collected the same year that the species was described."

Upon studying this autotype specimen sent by Dr. Ewing it was found that the illustration of *D. michaeli* did not agree with the description in the following particulars: the description calls for pectinate and flagelliform pseudostigmatic organs while the illustration figures barbed and rod-like pseudostigmatic organs. Thus the description is based on two species. This is borne out by the two habitats mentioned in the description: "In moss and under bark of logs." I am therefore restricting *D. michaeli* to the species with the pectinate, flagelliform pseudostigmatic organs, notogaster and leg segments bearing pectinate bristles; anal and genital covers separated by about one-half their length: and under *Belba jacoti* sp. n. the related species described below.

Belba jacoti sp. n.

Figures 2, 3, 4

DIAGNOSIS

Pseudostigmatic organ slightly shorter than cephalothorax, straight, not flagelliform, barbed. Notogaster with 18 stout, slightly curved smooth spines; surface of notogaster smooth. Legs moniliform, each joint with one or more stout curved and barbed bristles. Abdomen globular. Anal and genital apertures close together.

DESCRIPTION

Cephalothorax, as seen from above, pyriform, broad, sides tapering gradually to the blunt, conical rostrum; tectopedia invisible both from the dorsal and lateral views; surface smooth; two pairs rostral bristles, medium long; interlamellar bristles stout, of medium length; exopseudostigmatic bristles short and stout; pseudostigmata cup-shaped; pseudostigmatic organ rod-like, slightly expanded distally and barbed, then tapering to a fine point.

Abdomen high, circular, without forewaist spines; notogaster smooth, bearing 18 stout slightly curved bristles which in the adult are smooth, notogaster frequently carrying the cast skins of previous molts, the spines of the immature stages being barbed. The posteriormost bristles are well down on the notogaster; three short bristles each side near posterior margins.

Ventral plate with posterior edge slightly undulating. Anal aperture close to posterior edge of ventral plate, broadest posteriorly, tapering

slightly toward the anterior end, anal covers each with two long fine bristles, one near the anterior margin and one near the center. The postanal bristles long and fine; mesal postanal bristles between posterior margin of anal aperture and posterior edge of ventral plate; lateral postanal bristles near the anal aperture at the posterior angle, preanal bristles on the same transverse plane as the center anal cover bristle, six paramesal bristles located on a curving line from posterior of genital aperture to lateral edge of ventral plate, one bristle near center of ventral plate between coxa IV and genital aperture, four long bristles on a straight line from anterior margin of coxa IV to center of genital aperture, two long, fine bristles on a line anteriad of genital aperture, one long fine bristle near suture between cephalothorax and ventral plate (fig. 4). Genital aperture oval, genital covers with five well developed cover bristles placed as shown in figure 4, genito-thoracic suture not visible, three bristles on parasternum I arranged as in figure 4, the one near the lateral margin much longer than the other two, one bristle on parasternum II near coxae II (fig. 6).

Legs moderate in length, moniliform, each terminated by a well-developed, strongly curved hook, armed with one or more stout, curved, barbed bristles on all segments except the coxae. Legs IV the longest, legs III the shortest.

Legs I (fig. 6) longer than legs II. Coxae curved and slender at body, slightly larger at distal end with one bristle on dorsal surface. Femora with long spindle-shaped portion and much enlarged distal portion, one strong, barbed dorsal bristle, three outer lateral bristles, one about the center of the spindle portion and two on the enlarged portion, one ventral bristle near the distal end and one inner lateral bristle. Genuals squash-shaped with three stout barbed bristles, one dorsal, one outer lateral and one inner lateral. Tibiae shorter and larger than genuals, with five bristles, two dorsal, the major bristle on the outer lateral side, one ventral and one inner lateral bristle. Tarsi with enlarged portion near proximal end, with three bristles on dorsal face, three on outer lateral side, two on ventral face, and three on inner lateral side.

Legs II similar to legs I, but each segment slightly shorter. Coxae with one bristle on dorsal surface, femora with one dorsal bristle, three outer lateral, one ventral, and two inner lateral bristles. Genuals with three bristles, one dorsal, one outer lateral, and one inner lateral. Tibiae with one dorsal, one outer lateral, two ventral, and one inner lateral bristle. Tarsi with two dorsal bristles, four outer lateral bristles, four ventral bristles, and three bristles on inner lateral side.

Legs III shorter than legs II. Coxae greatly enlarged with two bris-

tles, one on outer lateral side and one on ventral face. Femora with one dorsal bristle, three outer lateral bristles, and two ventral bristles. Genuals with one dorsal, one outer lateral and one ventral bristle. Tibiae with one dorsal, one outer lateral and two inner lateral bristles. Tarsi with five outer lateral bristles, one on the enlarged portion, four ventral bristles, two on enlarged portion and two on shaft, three inner lateral bristles, one on the enlarged proximal portion, and two on the shaft, two dorsal bristles on the shaft.

Legs IV the longest. Coxae with a long cylindrical proximal portion and enlarged distal portion bearing one outer lateral bristle. Femora with one dorsal, two outer lateral, and one ventral bristle. Genuals with one dorsal, one outer lateral, and one ventral bristle. Tibiae with one dorsal, two outer lateral, and one ventral bristle. Tarsi with three dorsal bristles, one on the enlarged proximal portion, two on the shaft, two ventral bristles on the enlarged portion, and one on the shaft, and five outer lateral bristles.

Dimensions of three specimens given in microns are as follows:

	Greatest	*Average*	*Smallest*
Total length of body	479	441	413
Length notogastral plate	313	297	266
Breadth notogastral plate	266	266	266
Interlamellar bristle span	113	93	73
Camerostome to genital aperture	113	108	100
Length of genital aperture	73	67	66
Breadth of genital aperture	73	73	73
Genital aperture to anal aperture	27	24	20
Length of anal aperture	93	86	80
Breadth of anal aperture	73	70	66

Specimens studied:

Three specimens from oak leaves and moss, Neumans Lake, Gainesville, March 25, Slide nos. 55-D14, -D17, -D18; three specimens from leaves on beach, St. Augustine, April 1, Slide nos. 63-D7, -D25, -D26; one specimen from small leaves of *Tamola littoralis*, St. Augustine, March, Slide no. 34-D.

Holotype, Slide No. 55-D14.

Belba alachua sp. n.

Figure 7

DIAGNOSIS

Body less heavily chitinized than *B. jacoti*. Cephalothorax smooth, pseudostigmatic organ long, flagelliform, smooth. Notogaster with

eight smooth bristles on each side, without forewaist spines. All of the body and leg bristles smooth.

DESCRIPTION

Cephalothorax broad without ridges or other marks. Rostrum rounded with one pair of rostral bristles. Tectopedia not visible. Pseudostigmatic organ very long and slender, ending in fine thread. Exopseudostigmatic and interlamellar bristles close to pseudostigmata, smooth, stout, and slightly curved.

Abdomen ovate, notogaster smooth, bearing 16 smooth, curved bristles arranged as shown in figure 7, and with three short bristles on each side near posterior margin. Ventral plate not encroached upon by notogaster, margin smooth. Anal aperture close to posterior margin of ventral plate; broadest posteriorly, tapering very slightly toward the anterior end; anal covers each with three short, fine cover bristles. The postanal bristles are fine, of medium length; mesal postanal bristles located between anal aperture and posterior margin of ventral plate; lateral postanal located near posterior angle of the anal aperture; preanal nearer the lateral margin of the ventral plate than the anal aperture and posteriad of the anterior margin of anal aperture. Three paramesal bristles on a transverse plane from the center of the genital aperture to anterior margin of coxa IV. Tectopedia IV just visible from the ventral view as a triangular projection between legs III and IV. Three bristles anterior to genital aperture and coxa III. Another bristle just anteriad of coxa III. Genital aperture almost round, genital covers each with five short, fine bristles (fig. 7). Two bristles on cepholothoracic sternum; one each on parasterna I and II.

Legs moderate in length, moniliform, each terminated by a single well developed strongly curved hook, armed with one or more, stout, smooth, curved bristles on all segments. Legs IV the longest; legs II and III about equal in length, slightly shorter than legs I.

Legs I (fig. 7) longer than legs II. Coxae slender at the base, suddenly enlarged at juncture with femora, one bristle at distal end. Femora with slender spindle-shaped portion and much enlarged distal portion, one outer lateral bristle near proximal end, three on enlarged distal portion, one bristle on dorsal face, and one on the ventral face. Genuals shorter and smaller in diameter than the femora with one bristle on each of the four faces. Tibiae with two bristles on outer lateral side, major bristle and one other on dorsal face, one bristle each on inner lateral side and ventral face. Tarsi with four outer lateral bristles, two on the

enlarged portion and two on the distal part of the shaft; two dorsal bristles on the enlarged portion; five inner lateral bristles, two on enlarged portion and three on shaft; two ventral bristles on the shaft.

Legs II similar to legs I, slightly shorter. Coxae with a single long, curved bristle at distal end. Femora with two outer lateral bristles, one on the proximal portion and one on the enlarged portion; one dorsal bristle; two inner lateral bristles; no ventral bristles; Genuals with one bristle on each of the four sides. Tibiae larger than genuals with one bristle on each of the four sides. Tarsi with four outer lateral bristles, two on the enlarged proximal portion and two on the shaft; two dorsal bristles on the shaft; six inner lateral bristles, one on the enlarged portion and five on the shaft; two ventral bristles on the shaft.

Legs III (fig. 7) about the same length of legs II. Coxae gourd-shaped with two ventral bristles, one on the proximal slender portion and one on the distal enlarged portion; one outer lateral bristle. Femora with two outer lateral bristles; one dorsal bristle; one inner lateral bristle; one ventral bristle. Genuals with one bristle on each of the four sides. Tibiae with one ventral bristle; one outer lateral bristle; two dorsal bristles, one inner lateral bristle. Tarsi with one ventral bristle near distal end of the shaft; three outer lateral bristles on the shaft and one on the enlarged proximal portion; two dorsal bristles on enlarged portion and one on the shaft; six inner lateral bristles; two on the enlarged portion and four on the shaft.

Legs IV the longest. Coxae gourd-shaped, the proximal slender portion rather long, one ventral bristle at the extreme distal end. Femora with one ventral bristle; two outer lateral bristles; one dorsal bristle. Genuals with one bristle each on the outer lateral side, dorsal side, and inner lateral side. Tibiae with a major bristle similar to the major bristle of legs I developed on the outer lateral face, one dorsal bristle; two inner lateral bristles. Tarsi with one outer long lateral bristle on the proximal portion and two ordinary bristles on the distal portion; two dorsal bristles on the shaft; five inner lateral bristles, two on the enlarged portion and three on the shaft.

Dimensions of two specimens given in microns are as follows:

	Greatest	*Smallest*
Total length of body	386	386
Length notogastral plate	260	233
Breadth notogastral plate	246	233
Interlamellar bristle span	80	80
Camerostome to genital aperture	106	86
Length of genital aperture	73	66

	Greatest	*Smallest*
Breadth of genital aperture	73	66
Genital aperture to anal aperture	20	20
Length anal aperture	80	80
Breadth anal aperture	60	60

Specimens studied:

Three specimens from Magnolia, oak and grape leaves, Horticulture grounds, Gainesville, January 21, Slide Nos. 3-D3, and 3-D4.

Holotype, Slide no. 3-D4.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
LEESBURG, FLA.

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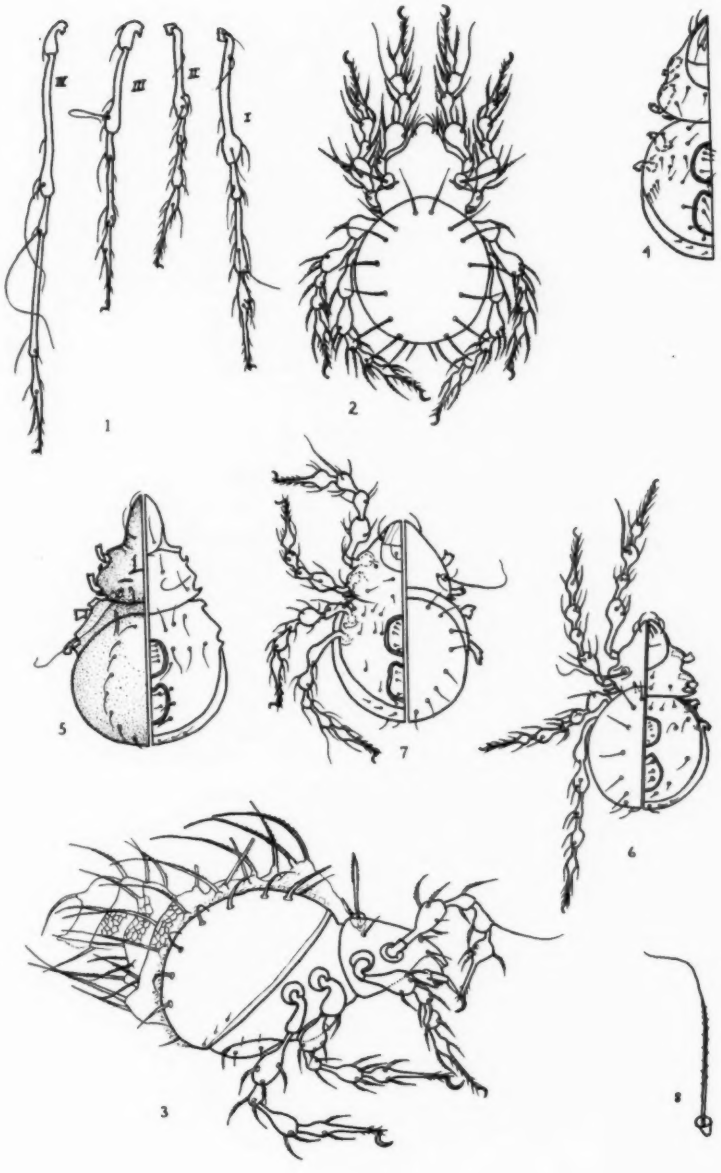
EXPLANATION OF PLATE 21

Fig. 1. Legs I, II, III and IV of *O. grossmani* sp. n.
Fig. 2. Dorsal view of *B. jacoti* sp. n.
Fig. 3. Side view of *B. jacoti* sp. n.
Fig. 4. Ventral view of *B. jacoti* sp. n.
Fig. 5. Dorsal and ventral view of *O. grossmani* sp. n.
Fig. 6. Dorsal and ventral view of *B. globifer florida* subsp. n.
Fig. 7. Dorsal and ventral view of *B. alachua* sp. n.
Fig. 8. Pseudostigmatic organ of *O. grossmani* sp. n.

PLATE 21



THE INCUBATION OF HEN EGGS UNDER INCREASED ATMOSPHERIC PRESSURE

By Bert Cunningham

A study of this nature must of necessity extend over a long period of time. The period in which one may secure fertile and viable eggs is restricted and the incubation period is relatively long. Furthermore, the usual control and recording instruments do not work satisfactorily under pressure, and new apparatus has to be devised for these purposes. Once a complete set up has been made there are multiplied possibilities that something will go wrong, and an experiment may have to be discarded because the electric current was off over night, or the air pumps were cut off for repair.

But these studies were not begun with a complete apparatus. The first experiments were conducted in 1927 with a pressure cooker, a bacteriological incubator, and an automobile pump. The results obtained were far from scientific, but they gave indubitable evidence that under the conditions of the experiments there was an acceleration of growth of the early chick embryo. To be sure this increased growth may have been due to higher humidity or it may have been due to elevated temperature, or finally it may have been due to the increased pressure which varied in a 24 hour period from 5 to 20 pounds.

A reserve air tank added later to the outfit aided in keeping the pressure up to 12–20 lbs. over night, but this did not materially change the results from those obtained when the pressure dropped to 5–8 lbs. during the night. The records of these early experiments were published in brief in the Journal of the Elisha Mitchell Scientific Soc. **42:** 188–192, 1927.

A large metal "incubator" built to contain a small commercial one was next added to the equipment. The smaller incubator did not prove successful and was replaced by a simple egg turner and the whole metal incubator was used as an incubating chamber. A description of this with its more recent additions has been printed in Science (**80:** 99–100, 1934). By the use of this improved apparatus it was not only possible to maintain a reasonably constant pressure, but much higher pressures could be used. This was limited by the pump to about 50 lbs., and would be limited by the incubator to about 75 lbs.

With the increase in pressure difficulties were experienced with electric thermo-regulators, heating elements, and fans. Ultimately heating elements and thermo-regulators were placed outside the incubator, and induction motor fans replaced the "brush" type within. Difficulties were also experienced with humidity controls. Finally one was devised using paper as an active agent and a mercury contact. This has proved very satisfactory.

The relative humidity had been calculated from the wet-dry bulb thermometers, but this has not been altogether satisfactory as the water evaporated from the "wet bulb" demands a considerable input and outflow of air to keep the humidity at the desired level. More recently a new device has been constructed so that readings may be made from time to time although no provision has been made for automatically recording them.

Early check experiments in which this pressure incubator was run at normal pressures indicated that it was inferior at that time to commercial incubators, and the following results therefore are the more striking.

Some 94 eggs were incubated in this apparatus at 20 lbs., 29 at 22 lbs., and 35 at 40–50 lbs. While the average weight of the embryos taken on the 7th day was not markedly different from the average weight of the 79 controls, the minimum and more especially the maximum were pushed far beyond the controls. The normal incubator gave a typical curve with 75 embryos weighing between 0.480 g. and 0.720 g., with three below the minimum of this range and one above the maximum. The curve was exceedingly flattened at 20 lbs. pressure. Of the 94 embryos but 40 lay within the above limits and there were 24 below 0.480 g. and 30 above 0.720 g. Furthermore the maximum weight at 7 days for the control embryos was less than 0.760 g., while that for the pressure was more than 1.000 g.

At 45 lbs. pressure only 16 of the 35 embryos weighed between 0.480 g. and 0.720 g. while 4 weighed less and 15 weighed more, the minimum weight being less than 0.360 g. and the maximum more than 0.960.

Up to this time readings of temperatures were taken several times daily as no provision had been made for continuous recording either of temperature or humidity. Since there is some evidence that extremes of humidity do affect developmental rates in the chick it was highly desirable to control and record the humidity as well as the temperature within the pressure incubator. A wet-dry bulb recording thermometer was added to the apparatus.

The control of humidity as well as the control of temperatures by electrical contact under pressure offered difficult problems, as did the use of the "brush type" fan and metal heating elements. These were solved by using induction motors on the fans, by placing the heating elements and their controls outside the incubator, and by the use of a special paper humidity control with a mercury trip contact.

The problem of the recording of humidity has not been so easily solved. The wet-dry bulb recording thermometers (corrected for pressures) have not been wholly satisfactory since at times the evaporation from the wet bulb increases the humidity beyond the desired point. This can be balanced of course by a flow of dry air through the incubator, which in turn required more water to be put into the incubator. More recently a mechano-electric indicator has been added. This is non-recording, and therefore entails considerable additional attention to the apparatus.

With the improved apparatus additional runs have been made, and during the present season a very satisfactory experiment has been completed in which the conditions of temperature and humidity in the pressure incubator paralleled that of the controls closely and the pressure alone was the variable.

Eggs of the White Leghorn secured from Mr. L. G. Cheek, a local poultry man, were used in this experiment. Sixty-eight eggs were set in the pressure incubator at 35 lbs. and seventy-six were used for controls. The eggs were weighed and matched before incubation. Of the 68 pressure eggs 54 were alive when opened on the 9th day, and gave an average weight of 2.07 g. while 68 of the 76 controls were alive and gave an average weight of 1.458 g. An additional control of 63 eggs run in Mr. Cheek's incubators produced 51 live chicks with an average weight of 1.494 g. This gave the pressure chicks an increase in weight of about 42 per cent over the controls.

From cumulative data, but more especially from this last experiment, there can be little doubt but that increased atmospheric pressure does accelerate the development of the chick embryo up to the 9th day of incubation.

The question can well be raised whether the increase in the weight of the embryo might not be due to imbibition of water, since it was found that pressure eggs lost water more slowly than normal eggs at the same relative humidity. The 54 pressure eggs in the above experiments, after 9 days of incubation, had lost on an average of 2.1 g. while the 68 controls had lost 3.01 g. per egg, this in spite of the fact that the

relative humidity was the same for each group. Comparable observations had been made the preceding year.

It seemed that a critical test of the question could best be made by determining the dry matter of the pressure embryo and comparing that with the controls. This had been done previously in our laboratory by Mr. Blair, and while his results are consistent with the findings of this year they are not so striking, largely, it is believed, because he used eggs incubated at a lower pressure. The 54 pressure embryos, and 68 normal embryos mentioned above were individually desiccated at 95°C. to a constant weight, which for the former averaged 1.162 g. and for the latter 0.709 g. or an increase of 61 per cent in favor of the pressure chicks. The pressure chicks during desiccation lost 55.6 per cent of the original weight while the normals lost but 48.6 per cent. With such evidence it would be difficult to doubt that the increase in weight of the embryo in the pressure incubator was due to additional tissue produced under the stimulus of the compressed air.

The accelerating effect of pressure does not appear to be co-extensive with embryonic life. Nor is the early advantage maintained. Since the death rate of pressure chicks mounts rapidly about the tenth or eleventh day, an experiment was performed in which eggs were incubated under 30–35 pounds pressure for 9 days, after which they were transferred to a normal pressure incubator and incubated for a total of 14 days. When the 38 embryos were removed and compared with normal embryos there was no significant difference in their weights. Similarly, eggs which have been incubated for 18–19 days under pressure when removed to a normal pressure incubator and allowed to hatch show no significant difference in the weight immediately after hatching; 23 newly hatched pressure chicks averaging 44.95 g. while 30 controls averaged 44.43 g.

There does seem to be on the other hand some effect on post-hatching growth. In one series of experiments 23 pressure chicks weighed 44.95 g. at hatching. Nineteen of these which survived to 21 days post-hatching averaged 113.8 g. while 47 controls averaged 44.6 g. at hatching and 45 survivors averaged 97.0 g. at 21 days.

This advantage in favor of the pressure chicks might be thought to be due to the survival under pressure of only the strongest and most virile chicks. Selecting the 9 largest males of the controls which averaged 117.3 g., and comparing them with the 9 surviving pressure males which averaged 130.5 g., it is evident that even under the best of condi-

tions for the controls the gain is 12.5 per cent; sufficient, it is believed, to be significant.

The mode of action is not so clear, but there are at least two possibilities which seem reasonable. It has been pointed out that increased humidity accelerates development. Now while the relative humidity in the pressure incubator has been held at the same level as in the control incubator, the water has not left the egg as rapidly and there is simulated a condition of high humidity. The increase in weight here however is much greater than can be obtained by optimum humidities. The critical test, i.e. regulating the humidity in the pressure incubator so that the water loss is equivalent to that under normal incubation, has not been made.

The second plausible explanation of the increased weight is that the metabolic level is raised by the increased oxygen pressure. This is not supported by any experimental evidence from this study. While there is no direct evidence to support this view it seems a little more reasonable to accept both as causal factors in the acceleration of growth in embryonic chicks under increased atmospheric pressure.

HETEROTHALLISM AND STERILITY IN ACHLYA AND OBSERVATIONS ON THE CYTOLOGY OF ACHLYA BISEXUALIS*

By JOHN ROBERT RAPER

PLATES 22–24

INTRODUCTION

Weston (1917) described a sexually sterile form of *Achlya* which produced heavy-walled resistant spores. The vegetative and sporangial development of the fungus agreed with other members of the genus, as *A. americana*, but in its further development the fungus differed from other Achlyas in that zoosporangium formation was followed normally by the production not of sexual organs, but of spherical heavy-walled resistant spores. The resistant spores averaged 110 μ in diameter, the size being apparently correlated with the vigor of the mycelium. In water the resistant spores germinated to form zoosporangia while in nutrient solutions mycelia were formed directly. Weston tried to induce the plant to form sexual organs by growing it in different chemical and nutrient solutions but without success.

Coker (1923) found and described what he considered to be the same plant and called it simply *Achlya* sp., form without oogonia. The resistant spores of Weston he considered as gemmae, but does not mention the fact that they are more heavy-walled than the gemmae. He was also unable to induce the formation of sexual bodies by varying the food supply and the external conditions.

Coker (1927) reported, from the unpublished notes of A. B. Couch, that heterothallism had been shown to exist in a form of *Achlya*, which he says, to all appearance, is the same plant as described in "The Saprolegniaceae" (1923) as *Achlya* sp., form without oogonia. He named the plant *A. bisexualis*.

The present study was undertaken to determine the extent of heterothallism in the genus *Achlya* and to investigate the cytology of the

* A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany.

resistant spores and the sexual organs of *A. bisexualis*. This study was suggested and made under the supervision of Dr. J. N. Couch and Dr. W. C. Coker to whom the writer wishes to express a sincere appreciation for all the help and suggestions given by them. The writer also wishes to thank Miss Ruby Rice, Miss Mary Vardell, E. V. Deans, and Leland Shanor for collections and cultures of sterile *Achlya*.

MATERIALS AND METHODS

Samples of 25–50 cc. of water from streams, springs, or ponds, along with some of the sediment, were collected and brought into the laboratory. Soil collections of 5–10 cc. were also used as a source of material.

The isolations were made in the following manner: The collections were poured into sterilized Petri dishes, and distilled water (which had previously been treated with animal charcoal, filtered, and sterilized in the autoclave) was added. Into each of these dishes were dropped two or three halves of boiled hemp seed. The fungal growths on the hemp seed were examined at intervals of about 24 hours after their appearance and a single hyphal tip of any *Achlya* which appeared to be sterile was transferred to a plate of maltose-peptone agar (maltose, 0.3%; peptone, 0.1%, agar, 2.0%). After 2–4 days' growth the periphery of the resultant mycelium was usually quite free from bacteria. From the outer edge of the radial growth a few hyphal tips were cut off in a small square of agar and this was transferred to a drop of sterile water. The hyphae in the agar grew out into the water for a short distance and produced sporangia at their tips. These, on maturing, liberated a large number of zoospores in 6–8 hours after being placed in the water. A small drop of this water, containing a number of spores, was then streaked on an agar plate. After allowing a few hours for the spores to germinate, the plate was examined under a dissecting binocular microscope and the position of isolated spores marked. A day after streaking the spores on agar, the mycelia resulting from their germination were large enough to enable one to cut out small squares of agar containing a number of hyphal tips from their periphery using the microscope and a sharpened spear-head needle. These squares were then transferred to sterilized Petri dishes where half a boiled hemp seed was placed on each and enough sterile water added to cover the bottom of the dishes, yet of insufficient quantity to float the seed from the agar. The growth on the hemp seed became visible to the naked eye after 4–8 hours. Cultures were washed frequently during the actively growing period, i.e., for the first four or five days after transfer.

Stock cultures of all the strains have been cultured in sterile water on hemp seed, and have been washed at intervals of 2–3 weeks, fresh hemp seed being added whenever necessary. They have also been carried through the procedure for isolation described above at intervals of about 6 weeks to insure their purity.

Cultures for crosses have been made by adding hemp seed to agar squares taken from a clean mycelium of the desired strain on nutrient agar. The young plants were washed daily until old enough to be used in the crosses, i.e., until zoosporangial production was well under way and the vegetative growth was approaching maturity. This stage of development was usually reached 2–4 days after transfer to the hemp seed. The pair to be tested was then placed in a sterilized Petri dish with their hyphal tips barely touching and enough sterile water was added to keep them wet but not enough to float them apart or too close together. In the latter case, subsequent observations would have been rendered much more difficult due to the density of growth in the region of contact of the two mycelia. After sufficient intermingling had taken place to cause the mycelia to hold together, enough additional water was added to make about 3–4 mm. in the bottom of the dish.

Early in this work it was only by repeated transfers through several changes of agar that material was obtained which was entirely free from bacteria. Later a method has been used by which the fungus can be completely isolated from its bacterial contamination with far less effort and in a fraction of the time formerly required. Very small glass beads (1/3–1/2 mm. in diameter) are fused to the edge of a small glass circle, such as is used in making a van Tieghem cell. The circle is then placed in the bottom of a sterilized Petri dish, the glass beads preventing the circle from resting on the bottom of the dish. The nutrient agar is then poured into the Petri dish to a depth which brings its surface well up on the sides of the glass circle.

After the agar has solidified the plate is inoculated by transferring a few hyphal tips, sporangia, or resistant spores to the area of agar lying inside the glass circle. As growth takes place, the hyphae grow down through the agar and some of them grow under the glass circle. In growing down through the agar, the bacteria are left at the surface of the agar inside of the circle and that portion of the mycelium which lies outside of the circle is free from bacteria. To test the absence of bacteria in this portion of the mycelium, small squares of agar, containing a number of hyphal tips, were cut off from the edge of the mycelium and each was placed in a small flask of maltose-peptone solution (strength

same as in the agar used for isolation). A control of a small square from a culture known to be contaminated was treated in an identical manner. In the flasks containing the squares to be tested no clouding of the liquid occurred, a delicate spherical mycelium forming from the inoculum in the agar. This method has been tried on a number of cultures of other species badly polluted with bacteria and in all cases so far has proved successful.*

STRAINS OF STERILE ACHLYA

During a period of 18 months approximately 500 collections have been made. From these collections 32 cultures of sterile *Achlya* have been isolated. Most of the collections were made from the vicinity of Chapel Hill, N. C.; additional collections, however, have been made at Murphy, N. C., Welcome, N. C., Maryville, Tenn., and Baltimore, Md. All of the sterile forms with the exception of three (Nos. 26 from Baltimore, Md., and 19 and 27 from Maryville, Tenn.) were isolated from collections from Chapel Hill and vicinity.

Of the 32 strains, 8 have been shown to belong to the female strain of *Achlya bisexualis*, 7, males of the same species, 12 are occasional hermaphroditic forms of *A. bisexualis*, but in the presence of a male plant give a typical female reaction. The remaining 5 cultures have given no reaction whatever when crossed with any of the other plants. Abbreviated descriptions of the strains follow:

Achlya bisexualis:

A. Female strain:

Mycelium rather large; hyphae moderately large and tapering; sporangia normal for the genus; cylindrical to oblong gemmae produced in abundance; numerous terminal resistant spores produced either singly or in chains (figs. 1, 2, 27, and 34).

B. Hermaphroditic-female strain:

Vegetative growth indistinguishable from that of female plant. Abortive oogonia are produced occasionally in small restricted areas of the mycelium, accompanied by undeveloped antheridia (fig. 29).

C. Male strain:

Growth more extensive but thinner than in female strain; hyphae more delicate; gemmae typically oblong. Small hyphae common; re-

* Since this paper was received for publication, a paper by Blank and Tiffney has appeared describing a method of preventing the growth of bacteria in cultures of *Saprolegnia* by ultra-violet rays (Mycologia **28**: 324. 1936).—Ed.

sistant spores always lacking. A large portion of the sporangia in older plants being of a pseudo-dictiosporangial type (figs. 3, 4, 5, 28, and 33).

Morphological differences between male and female strains: (1) Smaller size of the female; (2) longer but thinner hyphae of the male; (3) presence of pseudo-dictiosporangia in male; and (4) the presence of the resistant spores on the female and their absence on the male.

The five collections which have remained sterile are quite different from either of the three strains of *A. bisexualis* and apparently belong to four different species. An abbreviated description will be given of each strain.

Sterile Form 1. Small, slow-growing; densely matted. Hyphae stout at base, tapering sharply to tip. Sporangia in cultures 4–5 days old and older, often pseudo-dictiosporangial type. Gemmae produced by the segmentation of the vegetative hyphae, hence cylindrical (figs. 6 and 31). Two collections: 28 and 31.

Sterile Form 2. Growth thin and extensive; growth reaching approximately twice the size of the preceding form. Hyphae large. Sporangia normal for the genus. Gemmae formed by the septation of the vegetative hyphae and sometimes up to 1.15 mm. in length (fig. 30). A single collection: 30.

Sterile Form 3. Small thin mycelium; individual hyphae moderately large. Among the spores are frequently found large masses, measuring up to 40 μ, which result from the incomplete segmentation of the contents of the sporangium. A single collection: 32.

Sterile Form 4. Mycelium very thin and extensive, often reaching a diameter of 6 cm. in water culture on hemp seed; sporangia in young cultures are typical in form and dehiscence for the genus *Achlya*, but in older cultures practically all of the spores are liberated by the disintegration of the sporangial wall in a typical *Thraustotheca* manner (figs. 7, 32, and 35). A single collection: 29.

These strains are of some interest as they are the first collections of *Achlya* lacking sexual organs to be described which do not agree in all particulars with the original plant of Weston, which was doubtless a female plant of *A. bisexualis*. It is possible that these forms will eventually be shown to be sexual strains of other species of *Achlya*.

STUDIES IN HETEROTHALLISM

With the exception of the five most recently collected plants, 19, 27, 30, 31, and 32, all possible crosses have been made between the cultures. In the case of the five, each has been crossed with at least two males, two females, and each of the sterile plants.

The results of these crosses are shown in the two accompanying tables, the first being the results of pairings of true females with males, the second, the results of crosses between males and hermaphroditic-females, which in their reaction with males are identical with the true females.

TABLE I

Summary table of crosses between true females and males of A. bisexualis

FEMALE STRAINS	MALE STRAINS						
	21	22	23	24	25	26	2
1	II	–	–	I	–	–	0
5	III	I	I	I	–	–	0
9	I	–	–	–	II	–	0
10	–	–	–	II	–	–	II
15	II	–	–	II	–	–	0
16	II	–	–	II	–	–	0
18	I	–	–	II	II	–	II
19	0	0	0	0	II	0	III

TABLE II

Summary table of crosses between hermaphroditic-females and males of A. bisexualis

HERMAPHRODITIC-FEMALE STRAINS	MALE STRAINS						
	21	22	23	24	25	26	27
2	II	–	–	–	I	I	III
3	III	–	–	I	–	–	0
4	II	–	–	I	–	–	0
6	I	–	–	–	–	–	0
7	II	–	–	–	–	–	0
8	I	–	–	II	–	–	0
11	III	–	–	III	–	–	0
12	II	–	–	II	II	II	III
13	I	–	–	I	–	–	0
14	III	–	I	–	–	–	0
17	II	II	–	II	–	–	0
20	III	–	–	–	–	–	0

In the tables, the Roman numerals indicate the relative abundance of sexual organs found in the matings: "III" indicates the presence of numerous oogonia along the line of intermingling of the hyphae from the two mycelia (in no case have the oogonia been plentiful enough to show as a distinct line to the naked eye); "I" indicates the presence of very few sexual organs; and "II" is an intermediate grade. Minus signs

("—") mark those crosses in which no reaction occurred between the male and the female plants. In a number of cases (mainly with the two sexual strains most recently collected (19 and 27), no crosses have been made; zeros ("0") indicate such cases.

From a comparison of the results recorded in the two tables it is evident that the reaction between males and females was, at best, rather uncertain. Two males, 21 and 24, constantly gave better reactions than any of the remaining males with the possible exception of 27, for which only five of the possible twenty matings with female or hermaphroditic-female plants have been made. In those five crosses in which 27 has been used the reactions have been more nearly constant than has been the case when any other male was crossed with a number of females; also, in these same crosses the percentage of eggs which have matured has been definitely above the average for all crosses.

A marked variation in the sexual strength of certain females has also been evident. No female, however, seemed to be very constant in its reaction, but of the twenty, 5 and 12 have perhaps been the best; 19, from Maryville, Tenn., only used in a very few crosses, have in those crosses given relatively constant reactions.

The sexual reaction between male and female plants of *A. bisexualis* may be described as follows: in the region where the hyphae of the two mycelia intermingle, oogonia appear on the hyphae of the female plant. During their formation very small branches, the antheridial hyphae, arise from the main hyphae of the male plant and become applied to the sides of the oogonia (fig. 8). Also many of the antheridial hyphae twine around the vegetative hyphae of the female plant as well as the oogonial stalks, frequently forming complex gnarls of hyphae (fig. 9). A large number of antheridial branches are commonly found on each oogonium. The antheridia may be simple or branched and are frequently finger-like in shape, being applied to the oogonia by their sides or ends.

Most of the oogonia are borne on lateral branches of the main hyphae, but some terminal ones are commonly produced. Terminal resistant spores have been seen which functioned as oogonia, antheridia being applied to their sides and the contents dividing to form a number of eggs (fig. 10). This, however, is rare.

It may be said, however, that no matings could be called satisfactory. In all of the crosses the number of oogonia was somewhat less than hoped for, and the percentage of eggs which disintegrated before reaching maturity was exceedingly high. Even in the crosses which pro-

duced the greatest number of oogonia they were not plentiful enough to be visible to the naked eye as a line, a condition often seen in crosses of another heterothallic Oomycete, *Dictyuchus monosporus*, described by Couch (1926). Also a far smaller percentage of the eggs formed reach maturity in *A. bisexualis* than in that species, in which, however, not nearly all of the eggs mature in crosses made under cultural conditions.

A consideration of this inefficiency in producing large numbers of oogonia and germinable eggs brings certain important questions to mind. The foremost of these is whether or not the plant shows such lack of proficiency in nature. It is probable that it does not and if this sexual weakness only occurs in culture there are two plausible explanations: (1) the failure to reproduce in the laboratory the cultural conditions necessary for normal sexual development, and (2) the failure to collect perfectly compatible strains.

It is even quite possible that the male and female strains described, and with which this work has been done, do not belong to the same species, considering the fact that so very few eggs mature and that the sexual reaction in general is so weak. It may possibly be only a case of incomplete hybridization as in matings of strains of different species of the Mucorales, as was first demonstrated by Blakeslee (1904).

A further consideration is the fact that all of the male and female plants with the exception of three are from collections made from a very small area, approximately 20 square miles. In all probability a number of collections from a wider range would yield strains that would be more compatible with each other and in whose reactions the efficiency of sexual reproduction would be greatly increased.

CULTURAL VARIATIONS

Both male and female strains of *A. bisexualis* are exceedingly variable in culture; the female or hermaphroditic-female, however, shows greater variations than the male. The resistant spores, so common and characteristic of the female and hermaphroditic-female strains, are not produced in hot weather (their production never occurring above a temperature of 30°C). Also the occasional occurrence of sexual organs on the hermaphroditic-female plants suggested the possibilities of cultural conditions affecting their production.

In an effort to induce the hermaphroditic-female plant to produce oogonia regularly in culture and to study the effects of nutrients and salts on the production of resistant spores, no. 8 ♀, a typical plant of

that strain, was grown in 45 different solutions of haemoglobin, leucin, peptone, levulose, glucose, saccharose, potassium phosphate, monopotassium acid phosphate, di-sodium acid phosphate, sodium bisulphite, ammonium tartrate, calcium phosphate, ammonium nitrate, sodium chloride, and potassium nitrate. The variations in growth and production of gemmae and resistant spores were as great as expected. No correlation between the relative growth of the plant and the abundance of gemmae and resistant spores could be established, nor did the production of gemmae and resistant spores, as a rule, parallel each other.

In certain solutions whose dissolved content was as high as 0.25% or higher, numerous dictiosporangia were formed. Klebs (1899) showed that variation in sporangial dehiscence could be brought about by changes in the concentration of the medium. In one solution, 0.375% glucose, an interesting intercalary variation of the resistant spore was found, which consisted of a group of three bodies, the two end ones spherical and the larger central one barrel-shaped. In only two of the solutions were any oogonia produced and in these there were very few, formed only in small restricted areas as typical for the hermaphroditic-female plant when grown under more nearly normal conditions.

CYTOLOGICAL OBSERVATIONS

Material to be used for cytological preparations was killed and fixed in (1) Claussen's fixative or (2) chromic acid—formalin solution (chromic acid, 1.25 grams, formalin (commercial 40%) 10 cc., and water, 200 cc.) for 6–10 hours. It was then washed for 12 hours, dehydrated, cleared, imbedded in paraffin in the usual manner, and sectioned 7.5–10 μ in thickness. The stains used were: (1) Gram's iodine method as described by Couch (1932); (2) the same stain with a slight counterstain of eosin; and (3) Heidenhain's iron-alum-haematoxylin method. Gram's stain was the most satisfactory of the three.

1. Vegetative hyphae.

The wall of the vegetative hypha is thin, 0.3–0.7 μ. A thin layer of granular vacuolated cytoplasm everywhere lines the wall to a thickness of 2–7 μ and in which numerous nuclei are scattered at irregular intervals (fig. 11, a & b). The vegetative nucleus consists of a conspicuous nucleolus, an area of clear nuclear sap, and a delimiting nuclear membrane. Along the nuclear membrane lie a variable number of dark staining granules from which extend very delicate threads to the nucleolus. The nuclei range in size from 3.1–3.5 μ, the average being 3.3 μ.

2. Resistant spores.

The resistant spores are characterized by their spherical shape and thick hyaline wall which varies in thickness from 1.5 to 2.2 μ according to the age of the body. The contents consist of dense, granular, vacuolated cytoplasm and numerous scattered nuclei (as many as 250 having been counted in a single "spore"). The wall of the mature resistant spore is made up of two distinct layers: an inner layer surrounding the individual spore and an outer layer which surrounds the entire chain of spores, lying in all places in close contact with the inner layer. In addition to these there is what appears to be a layer of gelatinous secretion which accumulates on the exterior of the resistant spore as it ages (figs. 12, 13).

The nuclei of the resistant spore are identical with those of the vegetative hypha in structure and size.

A marked change occurs in the cytoplasm of the resistant spore after it has been cut off from the parent hypha. In the young stages the cytoplasm appears to be identical to that of the hypha, being granular and containing numerous small vacuoles. In a slightly older stage numerous, very small, spherical, darkly staining bodies have appeared. As the "spore" ages these granules enlarge, and eventually attain the size of the nuclei (fig. 14, a-d). These granules are probably food inclusions of some sort and give the same appearance as similarly staining granules which lie in the cytoplasm of the zoospore. At the same time another change is taking place. The cytoplasm which, at an early stage, was finely granular has become more coarsely granular and the definite limitations of the scattered vacuoles have been lost.

In the "spores" of all ages there are numerous small rod-shaped, straight or bent, and spherical granules which stain dark with Heidenhain's haematoxylin (fig. 14e). These are probably chondriosomes; however, no special technique to show these bodies has been used as a check.

What is the true nature and function of the resistant spores? This problem has been evident since these bodies were first discovered by Weston (1917), who considered them to be wholly vegetative in function as a compensation for the supposed lack of a sexual phase in the life history of the fungus. The fact that they occasionally behave as female sexual organs has been stated above, and it is conceivable that this is their primary function and that due to the heterothallic nature of the plant and the possibility of frequent isolation of the female from the male element they have developed the ability to remain as vegetative resting bodies when the male element is not at hand. In the event

of the two sexual strains coming in contact, in some cases, the contents probably undergo the various phenomena that takes place in the oogonium when its contents are differentiated into a number of uninucleate eggs. As the functioning of the resistant spore is so rare, we were not fortunate enough to get any cytological evidence as to what goes on in such cases. Although these bodies are originally formed in the same manner as similar structures of *Saprolegnia dioica* described by Walz (1870), they have been shown to exhibit greater versatility of function by occasionally acting as female sexual organs.

Cultures of a typical female having abundant "resistant spores" were tested to determine the resistance, if any, of the spores to desiccation. Small masses of hyphae and terminal spores were put on filter paper and allowed to dry in air. No spores could be germinated after the filter paper on which they were placed became dry. The spores are thus perhaps no more resistant to desiccation than vegetative hyphae or gemmae.

3. Gemmae.

The gemmae are obviously only accumulations of cytoplasm in portions of the vegetative hypha, as the wall of the gemma is no thicker that that of the hypha. The lining layer of cytoplasm, however, is much thicker and contains a proportionately greater number of nuclei than the hypha. The cytoplasm and nuclei appear to be identical with that of the vegetative hypha (fig. 15).

4. Sexual organs.

Due to the small number of sexual organs available for study, the cytological story here is incomplete in many details and the present account is considered as preliminary.

The oogonium, a spherical body, contains a peripheral layer of finely granular, vacuolated cytoplasm and 50–100 nuclei which lie in the cytoplasm in a more or less regular manner, the distance between nuclei usually being practically the same. Antheridia numbering from one to several lie in close contact with the oogonial wall (fig. 16).

The wall of the oogonium is somewhat thicker than that of the vegetative hypha, but not so thick as that of the resistant spore. It is smooth, unpitted, and averages 0.7–1.5 μ in thickness. The wall of the antheridium is exceedingly thin, certainly being no thicker than a hyphal wall.

The nuclei of the young oogonium are identical with those of the vegetative hypha, each consisting of chromatin granules, a nucleolus, a region of nuclear sap, and a delimiting membrane. The nuclei of the

antheridia are at this stage, and during the following changes, in a similar condition to those inside the oogonium. In an older stage a definite enlargement of the nuclei of both sexual organs occurs. The granules on the nuclear membrane become more conspicuous and a contorted structure, which is evidently the spireme of the early stage in mitosis, lies in the nuclear sap. The nucleolus is also considerably enlarged, and in some cases, apparently divided into two smaller darkly staining bodies (fig. 17). No change is apparent in the cytoplasm.

From the preparations which have been studied, it appears that the nuclear membrane now disappears. The spireme breaks transversely to form a number of distinct chromosomes (fig. 18). Most of the nuclei now begin to disintegrate, the chromosomes and nucleolus becoming much smaller and less distinct, a similar reaction taking place in the antheridium. A small number of nuclei in each oogonium, however, complete their mitotic division (fig. 19). Several mitotic figures have been seen in each of a number of oogonia.

A detailed study of a mitotic figure shows a typical spindle having a distinct dark centrosome at either pole and a number of chromosomes lying along the fibers between (fig. 20, a and b). The chromosomes are irregular in shape. The number of chromosomes could not be determined with certainty due to their exceedingly small size, but as many as eight have definitely been seen.

The formation of eggs in *A. bisexualis* is in all probability like that described by Trow (1899 and 1904), Mücke (1908), Claussen (1908), Davis (1903), and others for various species of the *Saprolegniaceae*, but unfortunately no stages have been found. However, eggs which had evidently recently been formed have been seen in large numbers. In the young egg the membrane is thin and numerous small vacuoles are scattered in the cytoplasm. The vacuoles here apparently represent the oil droplets in the living condition, the oil having been dissolved out in preparation by the clearing agents (figs. 21, 22). As the eggs mature these vacuoles coalesce to form larger vacuoles.

Numerous oogonia have been found which had one or more fertilization tubes extending into them from the accompanying antheridia (figs. 22 and 23). Every fertilization tube that has been observed has always contained a nucleus in its tip. The oogonial wall at the point of entrance of the fertilization tube is slightly raised.

It appears that fertilization takes place, although no cases were seen where the antheridial tube was clearly connected with the egg. A number of eggs, however, were seen which contained two nuclei (fig. 24),

one lying in the center of the egg, supposedly the female nucleus, and another at some distance from it. Other stages were seen which appeared to be fusion stages (fig. 25), but this could not be determined with assurance.

The mature, or nearly mature, egg is surrounded by a thick wall which is composed of two layers. The wall of the egg is strikingly similar to that of the resistant spore. There is, however, no sign of the outer "secretion" layer as in that body. There is a single large vacuole in the mature egg, it being, supposedly, the end result of the fusion of the many smaller vacuoles of the younger egg and represents the position of the eccentric oil drop seen in the egg in the living condition. The cytoplasm of the nearly mature egg lies around the vacuole, but it is accumulated into denser masses on the side opposite the eccentrically located vacuole. A single large nucleus lies near the center of the cell at the edge of the vacuole (fig. 26).

SUMMARY

1. A new method for the isolation of water fungi from bacterial contamination is described.

2. *A. bisexualis* is a relatively uncommon plant, only 27 plants having been found in 500 collections.

3. Plants of *Achlya bisexualis* are shown to belong to three morphologically distinct strains: male, female, and hermaphroditic-female. An abbreviated description is given of each strain and certain morphological differences between male and female strains are pointed out. Aside from the occasional production of abortive oogonia and antheridia by the hermaphroditic-female plants, this strain is indistinguishable from the female.

4. Four additional strains of sterile *Achlya* have been collected and are here briefly described. They are different from any of the three strains of *A. bisexualis* and are perhaps sexual strains of other heterothallic species of *Achlya*.

5. Vegetative variations of the hermaphroditic-female strain of *A. bisexualis* have been induced by the use of various solutions of nutrients and salts, but the regular production of sexual organs has not been accomplished.

6. A cytological study of the resistant spores of the female and hermaphroditic-female strains of *A. bisexualis* is given. The resistant spore, so far as structure is concerned, more nearly resembles the vegetative structures of the fungus than the oogonium. The latter was

thought probable because of the fact that the resistant spore occasionally functions as an oogonium.

7. Cytological observations on the sexual organs of *A. bisexualis* agree with accounts of work on other members of the *Saprolegniaceae*.

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PLATE 22

Achlya bisexualis

Figs. 1–5

Fig. 1. Resistant spores of female plant. ×42.
Fig. 2. Germinating resistant spore showing sporangium at tip of germination tube. ×75.
Fig. 3. Tip of pseudo-dictiosporangium of male plant. ×395.
Fig. 4. Stages in spore emergence from pseudo-dictiosporangium; cysts remaining in sporangium form a false net. ×795.
Fig. 5. Typical gemmae of male plant. ×65.

Achlya, sterile forms

Figs. 6–7

Fig. 6. Typical gemmae and normal *Achlya* sporangium of Sterile Form 1. ×42.
Fig. 7. Hyphal tip of Sterile Form 4, showing a normal *Achlya* sporangium and one whose wall is disintegrating in a typical *Thraustotheca* manner. ×115.

Achlya bisexualis

Figs. 8–29

Fig. 8. Young oogonium and antheridial branches. ×200.
Fig. 9. Young oogonium and oogonial stalk surrounded by antheridial hyphae, a phenomenon frequently seen in the species. ×42.
Fig. 10. A former resistant spore, the contents of which have become differentiated into a number of eggs, apparently serving the function of an oogonium. Optical section. ×42.
Fig. 11. Transverse section of a vegetative hypha. ×1197. Also vegetative nuclei showing detailed structure. ×2015.
Fig. 12. A chain of resistant spores in longitudinal section. ×180.
Fig. 13. A detailed study of the wall structure at the point where the two upper resistant spores of Fig. 12 are in contact. ×795.
Fig. 14. Portions of resistant spores shown in Fig. 12; *a* is the spore at lower end of chain; *b*, the second from the end, etc. Note progressive thickening of wall and "growth" of the dark-staining granules; *e* is a portion of another resistant spore of the same stage as *d*, but stained with Heidenhain's haematoxylin. ×795.

PLATE 23

Fig. 15. Longitudinal section of a gemma and adjacent portions of hypha. ×395.
Fig. 16. Young oogonium and four antheridial branches; nuclei of both sexual organs in resting stage. ×795.
Fig. 17. Slightly older oogonium than shown in Fig. 16; nuclei in early prophase of mitosis. ×795.
Fig. 18. Oogonium; nuclei in late prophase of division, the spireme having divided into a number of distinct chromosomes. ×795.
Fig. 19. Oogonium; six nuclei beginning to disintegrate; two others in late metaphase of mitosis. ×795.
Fig. 20. Detail of mitotic figures; *a* is the larger of the two mitotic figures of oogonium shown in Fig. 19; *b* is from another oogonium. ×2015.
Fig. 21. Young egg; membrane thin, numerous small vacuoles. ×795.
Fig. 22. Oogonial wall pierced by fertilization tubes. ×795.
Fig. 23. An oogonium into which a fertilization tube extends from one of the adherent antheridia; nucleus in extreme end of tube. Nuclei of two eggs in adjoining section. ×795.
Fig. 24. Fertilized egg; female nucleus in center and male nucleus a short distance away. ×795.
Fig. 25. A stage in the fusion of the male and female nuclei. ×795.
Fig. 26. Nearly mature egg surrounded by thick two-layered wall. The numerous vacuoles of earlier stages have coalesced to form a single large eccentric vacuole. ×795.

PLATE 24

Achlya bisexualis

Figs. 27–29

Fig. 27. Female plant. ×1.
Fig. 28. Male plant. ×1.
Fig. 29. Hermaphroditic-female plant. ×1.

Achlya, Sterile forms

Figs. 30–32

Fig. 30. *Achlya*, Sterile Form 2. ×1.
Fig. 31. *Achlya*, Sterile Form 1. ×1.
Fig. 32. *Achlya*, Sterile Form 4. ×1.

Achlya bisexualis

Figs. 33–34

Fig. 33. Pseudo-dictiosporangium of male plant. ×100.
Fig. 34. Resistant spores of female. ×100.

Achlya, Sterile form 4

Fig. 35. Sporangia of *Achlya*, dehiscing in a typical *Thraustotheca* manner. ×100.

PLATE 22

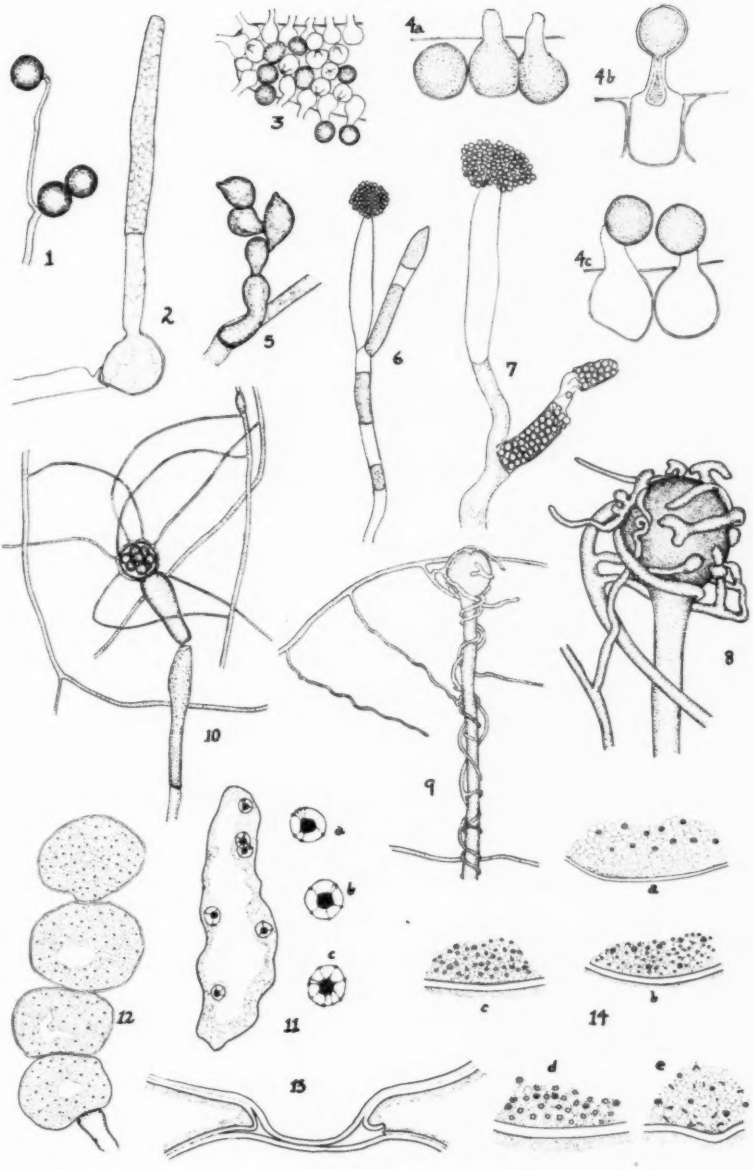


PLATE 23

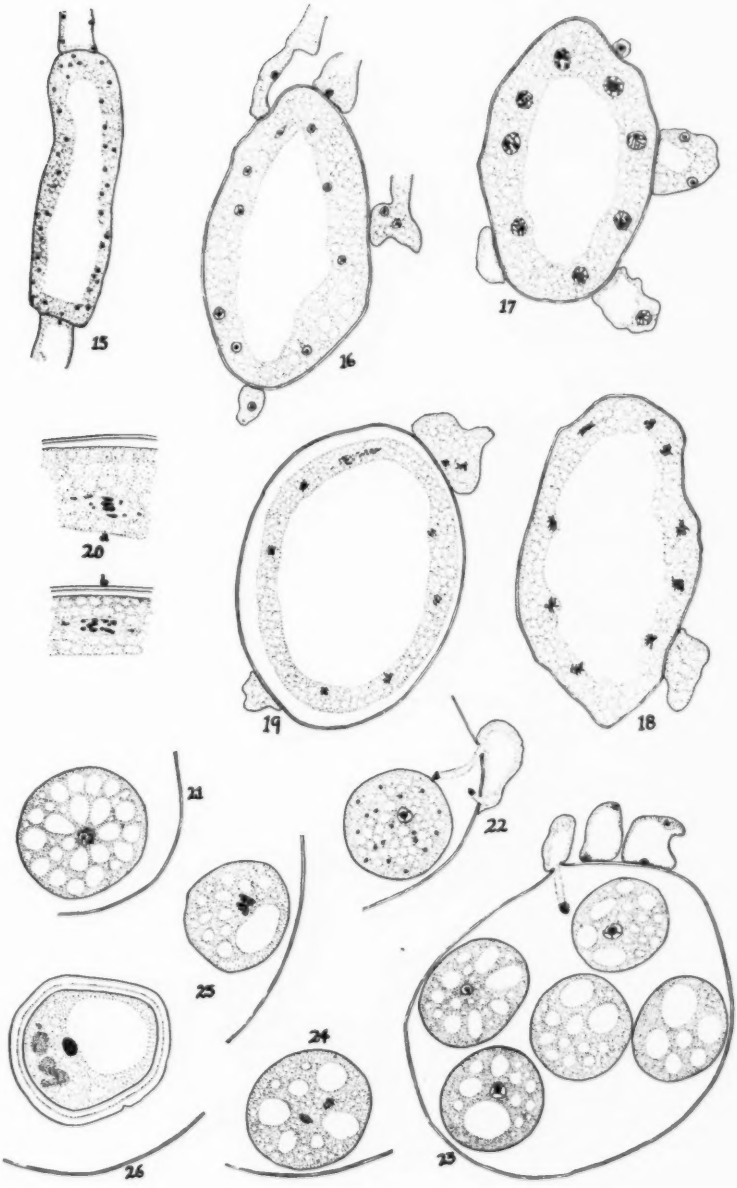
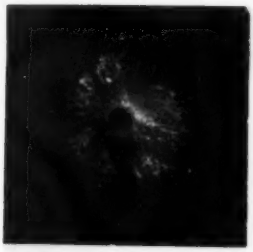


PLATE 24



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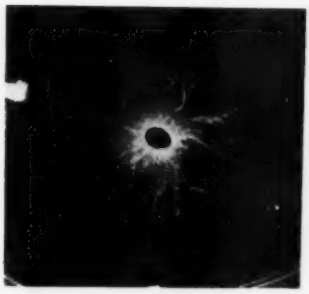

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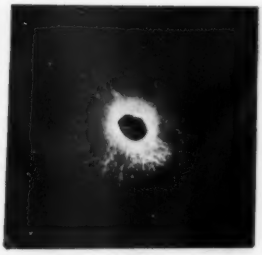

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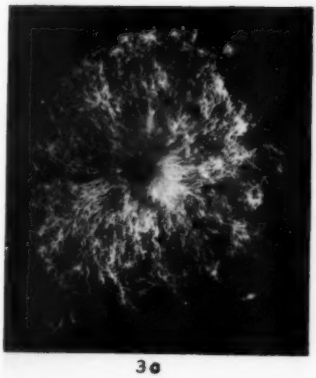

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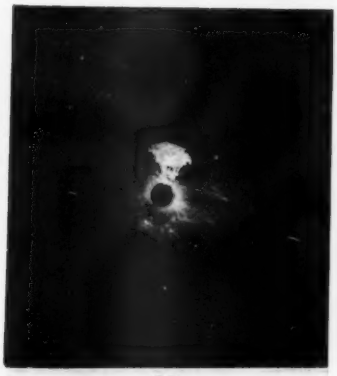

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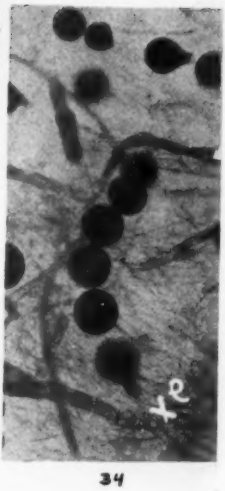


34

A NEW SPECIES OF RHIPIDIUM FROM MOUNTAIN LAKE, VIRGINIA

By VELMA D. MATTHEWS

PLATE 25

While examining a number of ripe huckleberry and *Amelanchier* fruits which had been lying for some time in the water of Mountain Lake, Giles County, Virginia, the writer noted a few fruits with very small clumps of fungus growth which proved to be a *Rhipidium* that is apparently undescribed. All the specimens of the new species were small, the longest main trunk (at least a thousand individual plants must have been examined) measured only 214 μ long, which is much shorter than the measurements given for *R. parthenosporum* Kanouse, which it resembles in the presence of the very short secondary branches which bear the sporangia and oogonia.

In the laboratory the sporangia formed zoospores only on a few occasions and then only after many attempts on the part of the writer to induce their formation. On one day several plants bearing many young sporangia were dissected out from the substratum about 9:30 A.M. and placed in a hanging drop of boiled lake water, which was changed several times during the day. By 8:00 P.M. many of the sporangia were forming zoospores, which escaped by the whole group pushing out in a mass, the individual spores then soon separating and swimming away as very active reniform, biciliate zoospores, containing a large vacuole and many large glistening granules. These zoospores in the hanging drop germinated within twelve hours by the formation of a tube, which branched very near its origin. Many attempts were made to grow this species in pure culture, but within the course of the three weeks available no growth of *Rhipidium* was found on the new cultures.

Oogonia mixed with the sporangia were rarely found on material just brought in, but developed in large numbers on plants kept in the laboratory for several weeks. These oogonia were similar to those in the other species of *Rhipidium* except that in many cases the thin wall had minute papillae. At the point where the antheridium comes in contact

with the oogonium a bulging of the oogonial wall was noted in several cases, which suggests a receptive papilla.

Rhipidium compactum n. sp. may be separated from all other species of *Rhipidium* so far described, except *R. parthenosporum*, by the very short branches which bear the sporangia and oogonia. It may be separated from *R. parthenosporum* by the shorter basal portion of the plant, by the presence of zoospores, by the smaller oogonia with papillae on the walls, by the sporangia and oogonia being borne in the same cluster, and by the presence of antheridia. Depauperate forms of *R. americanum* may bear sporangia on short pedicels, but the many plants found on ripe fruits at different times were very constant and apparently developing under suitable conditions and the antheridia were not borne on the stalk bearing the oogonium.

A detailed description of *R. compactum* n. sp. is given below:

Plants appearing on the substratum as small whitish pustules about 0.5–1 mm. in diameter. Individual plants composed of a main trunk, which may or may not be branched, a large number of short secondary branches on which the reproductive organs are borne, and a well developed system of large branched often lobed rhizoids, which may extend into the substratum up to a distance of about 725 μ. Main trunk unbranched or with as many as 8 large branches, 60–83 μ in diameter by 99–214 μ long, constricted slightly at the base where the rhizoids originate. Short secondary branches 9–42 μ (majority about 20 μ) long, from the large trunk, constricted at their point of origin bear the sporangia and oogonia, usually singly, occasionally two, very rarely three. Sporangia 2–10 on a main branch, very variable in shape on the same plant, globose to pyriform ones 33–36 x 42 μ, cylindrical ones 20–29 x 49–70 μ. Zoospores (rarely produced in the laboratory) reniform, biciliate, 6.4–8 x 11.2–12.8 μ, monoplanetic. Oogonia borne on same plant as sporangia and mixed with them, at times even arising from same short branch that bears a sporangium, spherical, 26–40 μ in diameter, wall thin, smooth or usually with minute papillae. Oospores one to an oogonium, 29–33 μ in diameter, wall at maturity sculptured and about 6.6 μ thick. Antheridia one to each oogonium forming a tube to the oosphere and borne on a long antheridial stalk arising from same plant but not from branch bearing the oogonium or in some cases perhaps from a separate plant.

On huckleberry and *Amelanchier* fruits in Mountain Lake at Mountain Lake, Giles County, Virginia, July and August 1936.

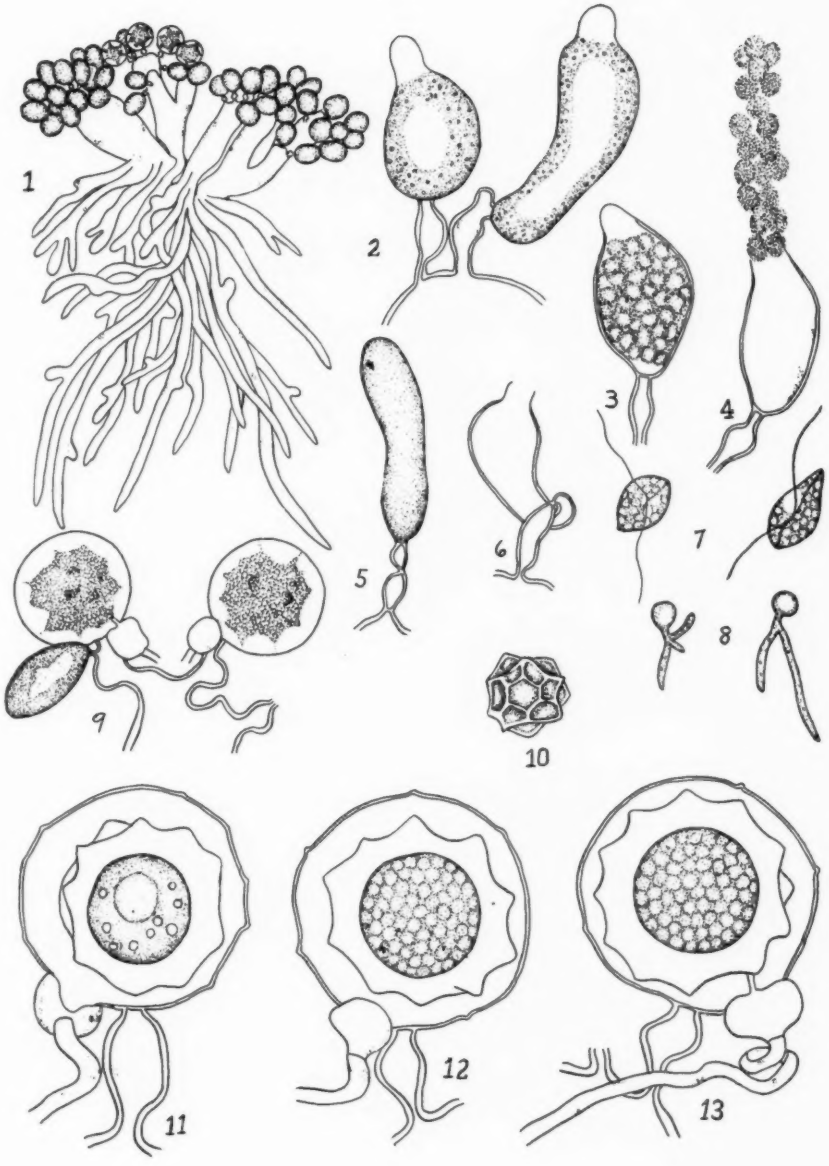
PLATE 25

Fig. 1. Cluster of plants showing habit of growth. ×111.
Fig. 2. Two young sporangia with large central vacuoles. ×460.

Fig. 3. Sporangium with zoospore origins. ×460.
Fig. 4. Sporangium with zoospores escaping. ×460.
Fig. 5. Young cylindrical sporangium borne on a branch constricted in the center. ×460.
Fig. 6. Empty irregular sporangium. ×460.
Fig. 7. Zoospores. ×1013.
Fig. 8. Germinating zoospores. ×460.
Fig. 9. Branch with a young sporangium and young oogonia. ×460.
Fig. 10. Mature oogonium. ×1013.
Fig. 11. Mature oospore. ×460.
Figs. 12–13. Oogonia with antheridia. ×1013.

COKER COLLEGE,
HARTSVILLE, S. C.

PLATE 25



THE GENUS CYPERUS IN NORTH CAROLINA

By Mildred Stites Reed

Plates 26–30

Although there are numerous species of the genus *Cyperus* in North Carolina and individuals of certain species are exceedingly abundant in most parts of the state, there seems to have been no especial work done on the group in this region. The genus has been treated only in general floras, such as Gray's (1908), Small's (1933), and Chapman's (1897), or in works on the *Cyperaceae* in other sections of the country, as Torrey's *Monograph of the North American Cyperaceae* (1836) and Britton's *List of the North American Species of Cyperus* (1886) in which several new forms were described. Little of this work is of recent date and is, therefore, open to certain corrections. Underwood (1932) made a study of the *Cyperaceae* of Tennessee. In 1934 Geise published a paper on the Indiana species of *Cyperus*. There has, however, been no study of the group in North Carolina or in any of the states which are geographically somewhat similar to it.

In view of these facts it has seemed desirable to make a study of the species of *Cyperus* in North Carolina in order to determine the number present, their habitats, the distribution in the state, and, since the genus is taxonomically a difficult one, to attempt to make a simple and usable key to the species.

Although it was impossible for the author to make collections of species of *Cyperus* from all sections, specimens from various herbaria have made it possible to work out fairly satisfactorily the state distribution, for there are representative collections from each of the three main geographical divisions of North Carolina: namely, the coastal plain, the piedmont, and the mountains.[1] As would be expected, the

[1] I wish here to acknowledge the consideration shown by the following herbaria in furnishing their North Carolina specimens of *Cyperus* for study: The United States National Herbarium, the New York Botanical Garden, the Missouri Botanical Garden, the University of North Carolina Herbarium, the University of West Virginia Herbarium, the Academy of Natural Science Herbarium, and the Duke University Herbarium. I wish also to express my appreciation to Dr. H. L. Blomquist, of Duke University, under whose direction this study was carried out.

more typically northern and western species of *Cyperus* are found in the mountainous western part of the state, while extensions of the more characteristically southern forms occur in the southeastern part of the state along the coast. Some species are, however, statewide in distribution.

The genus *Cyperus* belongs to the *Cyperaceae*, or sedge family, which is usually placed with the *Poaceae*, or grass family, in the order *Poales*. The common names used for the genus *Cyperus* are "galingale," "sweet rush," or, most often, merely "sedge." It is one of the largest genera of the *Cyperaceae*, having about 600 species widely distributed but occurring chiefly in the tropics and in the warmer parts of the temperate zones. The total number of species that occur within the United States is about 90, and in the course of this work 32 of these species have been identified as occurring in North Carolina. Among these are three species which apparently have not previously been reported as occurring in North Carolina: namely, *Cyperus Plankii* Britton, collected in Durham and Beaufort counties; *C. Engelmanni* Steud., collected in New Hanover County; and *C. sphacelatus* Rottb., found in "eastern North Carolina" apparently near Wilmington and probably growing on ballast there.

In studies which deal with the identities of species and their distribution over a statewide area, the worker usually finds that certain species have been reported as occurring in the state, which apparently are not really to be found there. Such has been the case in the course of this work. The confusion seems to be due in some cases to the unfortunate amount of synonymy which is present in this genus and in other cases to the confusion of one species with another that is similar in habit.

The first type of confused identity, that due to excessive and inaccurate synonymy, is well illustrated in the species *Cyperus ferax* L. C. Rich., *C. speciosus* Vahl., and *C. Michauxianus* Schultes. The specimens examined which were labeled as any of these species seemed to possess characteristics of all three, fitting one description as well as another. In fact, there seemed to be no distinguishing character on which even a varietal difference might be established, and the conclusion was reached that all of the North Carolina specimens, at least, belong to one species. This opinion has received confirmation in a recently published article by Fernald and Griscom of the Gray Herbarium (Rhodora, May, 1935), in which the species *C. speciosus, C. ferax,* and *C. Michauxianus* are discussed. Their conclusion is as follows:

"After attempting to find any character to distinguish these various plants, we are forced to treat them as a single wide-ranging species of which *Cyperus ferax* is the earliest name."

An excellent example of the other type of confused identity, that of mistaking one species for another that is similar in habit, was found in the case of *Cyperus rivularis* Kunth. and *C. diandrus* Torr. Both these species have frequently been identified as occurring in this state. The names seem to have been applied indiscriminately, for upon close examination there seemed to be no constant difference between plants labeled *Cyperus rivularis* and those labeled *C. diandrus*, except that the latter were usually larger than the former. Examination of specimens collected in other parts of the United States, however, showed that there is a great difference between the two. Both species have scales which at maturity are characteristically marked with red, but the manner in which they are marked seems to be distinct for each group. *Cyperus diandrus* has extremely thin, almost transparent scales, in which the red color seems to be concentrated at the margins, leaving the central portion of each side of the scale colorless. Those of *C. rivularis*, on the other hand, are firm and glossy and their color is spread over the entire scale or concentrated toward the center of each side of the scale rather than at its margins. In *C. diandrus* the style branches of every floret are exserted for two millimeters or more of their length, giving the spikelet as a whole a hairy or roughened appearance. *Cyperus rivularis*, however, has many of its styles included, although some of them may be exserted for a considerable distance. But there is no hairy appearance to the spikelet, and usually not even half of the scales have style branches extending conspicuously beyond them. In most taxonomic keys this difference in the length of the style branches is made the specific distinction between the two. When, however, *C. rivularis* is the only species present, as seems to be the case in North Carolina, difficulties arise because certain individuals of this one species seem to vary considerably in the length of the exposed style, and not having a specimen of the true *C. diandrus* for comparison, it is easy to see how a specimen with a few relatively long style branches might be classed as *C. diandrus* and another individual of the same species with shorter styles might be called *C. rivularis*. The evidence, from all the specimens examined in this study, indicates, therefore, that only *C. rivularis* is to be found here along with a more or less well-marked variety, *C. rivularis* var. *elongatus*.

Various species of the genus *Cyperus* appear in a number of types of habitats, ranging from extremely wet situations along lake shores or

in swamps where the lower part of the plant may even be immersed in water, to extremely dry sites in sand along the coastal plain or on xeric hillsides in the central or western parts of the state. There are, of course, all types of variation between these two extremes, and some species seem to be so adapted that they can live in any or all of these habitats. Most species of *Cyperus* seem to thrive on sandy and often acid soil. The majority are found in open places where there is an abundance of light, but several species are frequently found in woodlands or shaded situations.

The plants in the genus *Cyperus* are either annual or perennial. The roots are fibrous and in some cases have small, tuberlike swellings, the "tubers" being edible. The culms are triangular, erect or merely ascending, simple, and, in the case of perennial species, their bases are hardened into corms. Most of the perennials are also rhizomatous. The plants may be either solitary or tufted. Leaves are basal with linear-lanceolate blades, usually elongated but rarely reduced to membranaceous sheaths at the base of the stem. The inflorescence is a terminal spicate umbel, subtended by an involucre of from one to several, usually elongated, leafy bracts. The inflorescence may be either simple or compound, and the rays of the involucre, when present, are sheathed at the base and unequal in length. The spikelets are few to many-flowered, usually elongated, borne in spikes, panicles, clusters, or heads, and are flat or almost terete. Scales of the spikelets are two-ranked, conduplicate, and keeled. They may be either deciduous from the rachilla or persistent until after it has fallen from the rachis at maturity. The rachilla is often winged. The flowers are perfect and are without a perianth or perianth bristles. The stamens are from one to three in number. The ovary is superior, and its style may be either two- or three-cleft and is deciduous. If the style is two-cleft, the achene is lenticular; in species in which the style is three-cleft the achene is three-angled. In all cases, however, the achene is without a beak or tubercle.

In this state some species of *Cyperus* begin flowering by the middle of June, and one species, *C. rotundus*, has been collected in flower as late as the first of November. The genus as a whole is, however, a typically late summer plant in time of flowering. Best specimens should, therefore, be collected after this time, as most of the species fruit after the middle of August, and usually the mature fruit is necessary for the correct identification of a species.

The manual most used for identification in the course of this work

and upon which the nomenclature is to a great extent based is Small's *Manual of the Southeastern Flora*. Other manuals used include *Gray's Manual* (ed. 7), Britton and Brown's *Illustrated Flora of the Northern States and Canada* (ed. 2) and Chapman's *Flora of the Southeastern United States* (ed. 3).

The following key is as original and practical as it has been possible to make it, but in some instances the diagnostic distinctions are based on those used in Small's key to the genus *Cyperus*.

For accurate identification of any species of *Cyperus* it is necessary to have a specimen with mature fruits. The following key is based upon the characteristics of mature plants and mature fruits. If an attempt is made to identify an immature specimen, differences in color and length of parts should be taken into consideration, young spikelets and scales usually being green and all parts of the young plants being much smaller than the corresponding parts of a mature specimen.

KEY TO THE SPECIES

1. Achenes lenticular, their styles 2-cleft; plants annual.
2. Inflorescence distinctly terminal; spikelets strongly flattened; achenes laterally compressed.
3. Scales without scarious margins or with very narrow ones along the sides; achenes much shorter than the scales.
4. Spikelets obtuse to acutish, thick and rigid, yellow or red; rachilla wingless or very inconspicuously winged; achenes suborbicular or broadly obovoid.
5. Spikelets yellow or greenish, 1–2.5 mm. wide; mature achenes suborbicular, black, and shining ..... 1. *C. flavescens*
5. Spikelets red or variegated with red, 2.5 mm. or more in width; mature achenes grayish brown, broadly obovoid.
6. Spikelets 5–12 mm. long; scales 2 mm. long ..... 2. *C. rivularis*
6. Spikelets 15–25 mm. long; scales about 2.5 mm. long 2a. *C. rivularis* var. *elongatus*
4. Spikelets sharply acute, brown to greenish brown, not thick; rachilla somewhat winged; achenes linear to narrowly obovoid.
7. Scales 2.5–3 mm. long; achenes light brown, about 1.5 mm. or somewhat less in length ..... 3. *C. filicinus*
7. Scales 2 mm. or less in length; achenes dark brown or gray, 1 mm. or less in length.
8. Spikelets 1.5 mm. or more in width; mature achenes dark brown, narrowly obovoid ..... 4. *C. microdontus*
8. Spikelets about 1 mm. in width; mature achenes dark gray, linear to linear-ellipsoid ..... 5. *C. paniculatus*
3. Scales with broad scarious margins at the tips; achenes as long as the scales ..... 6. *C. sabulosus*
2. Inflorescence appearing lateral; spikelets subterete; achenes dorsally compressed ..... 7. *C. laevigatus*

1. Achenes 3-angled, their styles 3-cleft; plants annual or perennial.
9. Spikelets flattened.
10. Scales 3 mm. or less in length, deciduous from the rachilla at maturity.
11. Scales ending in long, subulate, recurved awns; achenes wedge-shaped ..... 8. *C. inflexus*
11. Scales acute, obtuse, or mucronate, not ending in recurved awns; achenes not wedge-shaped.
12. Spikelets broadly ovate, in dense, capitate clusters; stamens 1.
13. Scales nearly straight; achenes 1.5 mm. long, narrowly ellipsoid, about ½ as long as the scales ..... 9. *C. virens*
13. Scales curved; achenes 1 mm. long, linear, about as long as the scales ..... 10. *C. pseudovegetus*
12. Spikelets linear or ellipsoid, digitate or spicate; stamens more than one.
14. Spikelets lax in soft, plume-like, elongated spikes; achenes as long as the scales ..... 11. *C. Iria*
14. Spikelets rigid in stiff clusters or spikes; achenes much shorter than the scales.
15. Spikelets in digitate clusters.
16. Spikelets green or yellow; achenes 1 mm. or more in length ..... 12. *C. compressus*
16. Spikelets red or variegated with red; achenes less than 1 mm. long.
17. Culms weak; leaves usually reduced to sheathing scales at the base of the culm; spikelets less than 1 mm. wide ..... 13. *C. Haspan*
17. Culms rigid; leaves with elongated blade; spikelets 1.5 mm. or more in width.
18. Spikelets 4–15 mm. long; 3–4 mm. wide; scale tips not closely appressed, strongly mucronate ..... 14. *C. dentatus*
18. Spikelets 8–20 mm. long, 2–2.5 mm. wide; scale tips more closely appressed and not so strongly mucronate as in the species ..... 14a. *C. dentatus* var. *ctenostachys*
15. Spikelets in definite stiff spikes.
19. Spikelets red or variegated with red.
20. Spikelets less than 1 mm. wide; scales distant, truncate at the tips ..... 15. *C. distans*
20. Spikelets 1.5 mm. or more in width; scales imbricated, obtuse or acute.
21. Scales with very broad green keels, their sides green or yellow and variegated with red ..... 16. *C. sphacelatus*
21. Scales with narrow, bright green keels; their sides dark purple-red ..... 17. *C. rotundus*
19. Spikelets yellow, brown, or chestnut.
22. Spikelets few, distant in broad, stiff spikes; scales 2–3 mm. long; achenes brown.
23. Spikelets 2–3 mm. wide ..... 18. *C. esculentus*
23. Spikelets 1 mm. or less in width ..... 18a. *C. esculentus* var. *angustispicatus*
22. Spikelets many in dense, cylindric spikes; 1 mm. or less in width; scales 1–1.5 mm. long; achenes pale ..... 19. *C. erythrorhizos*
10. Scales 4–5 mm. long; either the scales deciduous from the rachilla at maturity, or the rachilla deciduous from the rachis, or both deciduous.

24. Umbel simple or compound; spikelets 8 mm. or more in length; 7 or more flowers on a spikelet.
25. Scales closely appressed; sheaths of the rays deeply 2-toothed.
26. Spikelets 8–18 mm. long; 7–12-flowered.
27. Rays 2–10 cm. long.....20. *C. strigosus*
27. Rays 20–30 cm. long.....20a. *C. strigosus* var. *elongatus*
26. Spikelets 20–30 mm. long, 10–15-flowered
20b. *C. strigosus* var. *robustior*
25. Scales lax and spreading at maturity; sheaths of the rays obscurely 2-toothed or truncate.....20c. *C. strigosus* var. *stenolepis*
24. Umbel very compound; spikelets 6–13 mm. long, 4–6-flowered
20d. *C. strigosus* var. *compositus*
9. Spikelets not flattened, terete or subterete; rachilla deciduous from the rachis at maturity, the scales persistent on the rachilla.
28. Scales distinctly imbricated; spikelets deciduous as a whole above the lowest pair of scales.
29. Spikelets about 1 mm. wide or less; subulate to linear-subulate; achenes 2.5 or more times as long as broad, linear to linear-ellipsoid.
30. Spikelets subulate; scales closely appressed and imbricated.
31. Spikelets distant in loose, elongated spikes.....21. *C. refractus*
31. Spikelets crowded into short-capitate or narrowly cylindric heads.
32. Heads obovoid or oval; at least some of the spikelets reflexed.
33. Culms rough, at least near the top; heads strongly obovoid; practically all the spikelets reflexed.
34. Rays rough; involucral bracts shorter than the umbel; heads greenish, short-turbinate.....22. *C. retrofractus*
34. Rays smooth; the longer involucral bracts surpassing the umbel; heads golden-brown at maturity, elongated obovate-cylindric
23. *C. dipsaciformis*
33. Culms smooth; heads oval; usually only the lower spikelets reflexed.....24. *C. lancastriensis*
32. Heads cylindric or globose.
35. Heads cylindric.
36. Sheaths of the rays distinctly 2-toothed; spikelets 2–4 mm. long, 1–2-flowered.....25. *C. Torreyi*
36. Sheaths of the rays truncate or mucronate; spikelets 5–7 mm. long, 3–5-flowered.....26. *C. Plankii*
35. Heads globose.....27. *C. ovularis*
30. Spikelets linear or linear-subulate; scales rather loosely appressed, somewhat imbricated; heads globose.....28. *C. globulosus*
29. Spikelets over 1.5 mm. wide, linear to linear-ellipsoid; achenes not more than twice as long as broad, broadly elliptic.
37. Culms slightly roughened on the angles near the top, rigid; spikelets in loose, cylindric spikes; rachilla broadly winged.....29. *C. tetragonus*
37. Culms smooth, filiform, sometimes lax; spikelets in globose, capitate clusters; rachilla narrowly winged or wingless.....30. *C. filiculmis*
28. Scales distant or very slightly imbricated; spikelets breaking up into 1-fruited joints at maturity.

38. Scales of the spikelet remote, their tips distant from the bases of those above them on the same side by about half the length of the scale; achenes narrowly linear-ellipsoid.....31. *C. Engelmanni*

38. Scales of the spikelets only slightly distant from those above them or somewhat imbricated; achenes ellipsoid or ellipsoid-obovoid...32. *C. ferax*

LIST OF SPECIES WITH HABITATS AND DISTRIBUTION

1. *Cyperus flavescens* L. Sp. Pl. p. 46. 1753. (Fig. 4)
Damp, usually sandy soils, in low grounds, sun or shade. Widely distributed in western and central sections and to some extent in the southeastern part of the state. July–October.

2. *Cyperus rivularis* Kunth. Enum. Pl. ii:6. 1837. (Fig. 10)
Wet or moist, usually sandy soils. Occurring in practically all sections of the state. August–November.

2a. *Cyperus rivularis* Kunth. var. *elongatus* Boekl. Linnaea xxxvi. 1870. (Fig. 9)
Habitat and range with the species. August–November.

3. *Cyperus filicinus* Vahl. Enum. ii:332. 1806. (Fig. 3)
On or very near the seashore, on sand or in brackish marshes. Eastern North Carolina. August–October.

4. *Cyperus microdontus* Torr. Ann. Lyc. N. Y. iii:225. 1836. (Fig. 5)
Wet or moist, sandy soils. Widely distributed in the eastern and central parts of the state. August–November.

5. *Cyperus paniculatus* Rottb. Descrip. & Icon. p. 40. 1773. (Fig. 6)
(*C. Gatesii* Torr.)
Low, wet ground near the coast. Found only in the southeastern part of the state. Rare. August–October.

6. *Cyperus sabulosus* Mart. and Schrad. Mart. Fl. Bras. **21**: 10. 1842. (Fig. 1)
(*C. flavicomus* Michx.)
Damp to fairly dry, usually sandy soil. Common on cultivated ground. One of the most common species in the central part of the state. July–November.

7. *Cyperus laevigatus* L. Mant. ii: 179. 1771. (Fig. 2)
Sandy soil near the seashore. Found only in the southeastern portion of the state. September–October.

8. *Cyperus inflexus* Muhl. Desc. Gram. p. 16. 1817. (Fig. 14)
Damp or wet, sandy soil, often on lawns or cultivated ground. Found in the central portion of the state. July–October.

9. *Cyperus virens* Michx. Fl. Bor. Amer. **1**: 28. 1803. (Fig. 11)
Sandy soil, moist to fairly dry sites. Found only on the coastal plain in the southeastern part of the state. Rather rare. July–October.

10. *Cyperus pseudovegetus* Steud. Syn. Pl. Cyp. p. 24. 1855. (Fig. 12)
(*C. calcaratus* Nees.)
Wet, sandy soils in marshes or on river banks. Common in the eastern and central parts of the state.

11. *Cyperus Iria* L. Sp. Pl. p. 45. 1753. (Fig. 20)
Low, wet ground or marshes, particularly on cultivated ground. Found in the central part of the state. September–November.

12. *Cyperus compressus* L. Sp. Pl. p. 46. 1753. (Fig. 13)
Moist to dry, usually sandy soil. A common weed on lawns and cultivated ground. Found in the central and eastern section of the state. July–November.

13. *Cyperus Haspan* L. Sp. Pl. p. 45. 1753. (Fig. 23)
Very damp or wet soils in swamps or wet woodlands. Found in the eastern and east central parts of the state. July–October.

14. *Cyperus dentatus* Torr. Fl. N. and Mid. U. S. **1**: 61. 1824. (Fig. 18)
Moist to fairly dry, sandy soils. Found only in the southeastern part of the state. Not common. August–October.

14a. *Cyperus dentatus* Torr. var. *ctenostachys* Fernald. Rhodora viii: 126. 1906. (Fig. 19)
Usually found associated with the species. August–October.

15. *Cyperus distans* L. f. Suppl. p. 103. (Fig. 21)
Wet woods or swamps in the southeastern part of the state. July–September.

16. *Cyperus sphacelatus* Rottb. Descr. Nov. Pl. **26**: Prog. 21. 1773. (Fig. 15)
Found in the southeastern part of the state along the coast where it was probably growing on ballast. Extremely rare. Only one specimen has been collected, but it is unmistakable. August–September.

17. *Cyperus rotundus* L. Sp. Pl. p. 45. 1753. (Fig. 29)
(*C. hydra* Michx.)
Moist to fairly dry habitats. A common weed on lawns. Found in the central portion of the state. July–November.

18. *Cyperus esculentus* L. Sp. Pl. p. 45. 1753. (Fig. 26)
(*C. phymatoides* Muhl.)
Moist soils, usually in open fields. Often a troublesome weed in cultivated ground. Occasionally cultivated as a stock food because of its edible "tubers." Statewide in distribution. July–October.

18a. *Cyperus esculentus* L. var. *angustispicatus* Britton. Bull. Torr. Bot. Club **13**: 211. 1886.
Its range and habitats are those of the species. July–October.

19. *Cyperus erythrorhizos* Muhl. Descr. Gram. p. 20. 1817. (Fig. 27)
Damp, sandy soils along river banks or in marshes. Found in the central and southeastern portions of the state. August–October.

20. *Cyperus strigosus* L. Sp. Pl. p. 47. 1753. (Fig. 30)
Moist to dry soils. Common in cultivated land. Widely distributed in all parts of the state. July–October.

20a. *Cyperus strigosus* L. var. *elongatus* (Torr.) Britton. Bull. Torr. Bot. Club **13**: 212. 1886.
Found in the central part of the state in habitats similar to those of the species. July–October.

20b. *Cyperus strigosus* L. var. *robustior* Kunth. Enum. ii: 88. 1837. (Fig. 7)
Its range and habitats are with those of the species. July–October.

20c. *Cyperus strigosus* L. var. *stenolepis* (Torr.) Kukenthal. Fedde, rep. Spec. Nov. xxiii: 189. 1926. (Fig. 16)
This variety is usually found in swamps or low grounds but sometimes

occurs on drier sites. (The reduction of this usually recognized species to the rank of a variety is based upon an examination of the type specimen (Curtis) at the Herbarium of the New York Botanical Garden.)

July–October.

20d. *Cyperus strigosus* L. var. *compositus* Britton. Bull. Torr. Bot. Club xiii: 212. 1886. (Fig. 8)

Distribution and habitats similar to those of the species. July–October.

21. *Cyperus refractus* Engelmann ex Boekl. Linnaea xxxvi: 369. 1869–1870. (Fig. 24)

Fairly moist to dry soils, in sun or shade, usually in woods or dry fields. Found mostly in the western and west central portions of the state.

July–September.

22. *Cyperus retrofractus* (L.) Torr. A. Gray Man. p. 519. 1848. (Fig. 25)

Dry, usually sandy soils, in woods or fields. Statewide in distribution.

July–October.

23. *Cyperus dipsaciformis* Fernald. Rhodora viii: 127. 1906. (Fig. 28)

Dry soils, usually in shady sites. Statewide in distribution.

June–October.

24. *Cyperus lancastriensis* Porter. A. Gray Man. p. 555. 1848. (Fig. 22)

Damp to dry soils, particularly in open fields. Found in the central and western parts of the state. July–September.

25. *Cyperus Torreyi* Britton. Bull. Torr. Bot. Club vii: 48. 1880. (Fig. 17)

(*C. cylindricus* (Ell.) Britton.)

Moist to fairly dry, sandy soils. Found in all parts of the state.

June–September.

26. *Cyperus Plankii* Britton. Small's Fl. S.E.U. S. p. 172. 1913. (Fig. 31)

Moist soils, particularly in open fields. Collected in the central and eastern parts of the state. July–October.

27. *Cyperus ovularis* (Michx.) Torr. Ann. Lyc. N. Y. iii: 278. 1836. (Fig. 32)

Moist to dry habitats, in fields or on stream banks. Common on cultivated ground. Widely distributed in the central and western parts of the state. Rather rare in the east. June–October.

28. *Cyperus globulosus* Aubl. Pl. Guian. i: 47. 1775. (Fig. 35)

(*C. echinatus* (Ell.) Wood., *C. Baldwinii* Torr.)

Rather dry, sandy woods or fields. Collected in eastern and central portions of the state and found as far west as Rowan County. July–October.

29. *Cyperus tetragonus* Ell. Sketch i: 71. 1821. (Fig 33)

Sandy soils, often in woodlands. Found only in the southeastern part of the state along the coast. July–October.

30. *Cyperus filiculmis* Vahl. Enum. ii: 328. 1806. (Fig. 36)

Dry, usually sandy soils. Statewide in distribution. June–October.

31. *Cyperus Engelmanni* Steud. Syn. Pl. Cyp. p. 47. 1885. (Fig. 34)

Collected in the eastern part of the state. Probably adventive. Rare. August–October.

32. *Cyperus ferax* L. C. Rich. Act. Soc. Hist. Nat. i: 106. 1792. (Fig. 37)

(*C. speciosus* Vahl., *C. Michauxianus* Schultes.)

Moist, usually sandy soils in swamps and along the streams and lakes. Found only in the eastern part of the state. August–October.

EXCLUDED SPECIES

The following species have been reported by collectors and writers from various parts of the state, but because they have not been observed in any of the collections studied, the existing data have not been considered sufficient to validate their inclusion in this paper.

Cyperus cayennensis (Lamarck.) Britton., reported from Currituck County by McAtee.

Cyperus repens Elliott, reported by Torrey as being collected by Muhlenberg in North Carolina.

Cyperus leptos Schultes, reported by Torrey as being collected by Curtis in New Hanover County.

Cyperus hystricinus Fernald, reported by McAtee from Currituck County.

Cyperus diandrus Torr, reported by various collectors from North Carolina but not observed among the specimens studied.

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DEPARTMENT OF BOTANY,
DUKE UNIVERSITY,
DURHAM, N. C.

PLATE 26

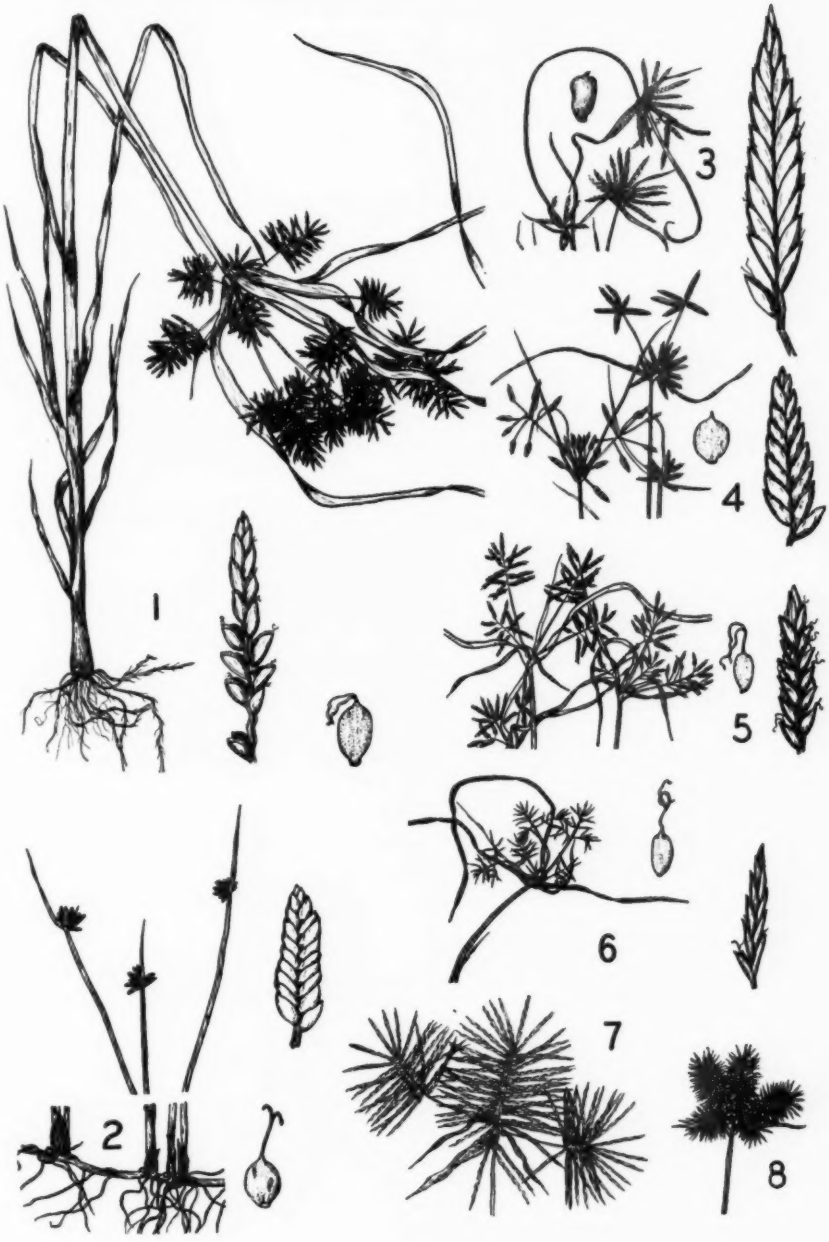
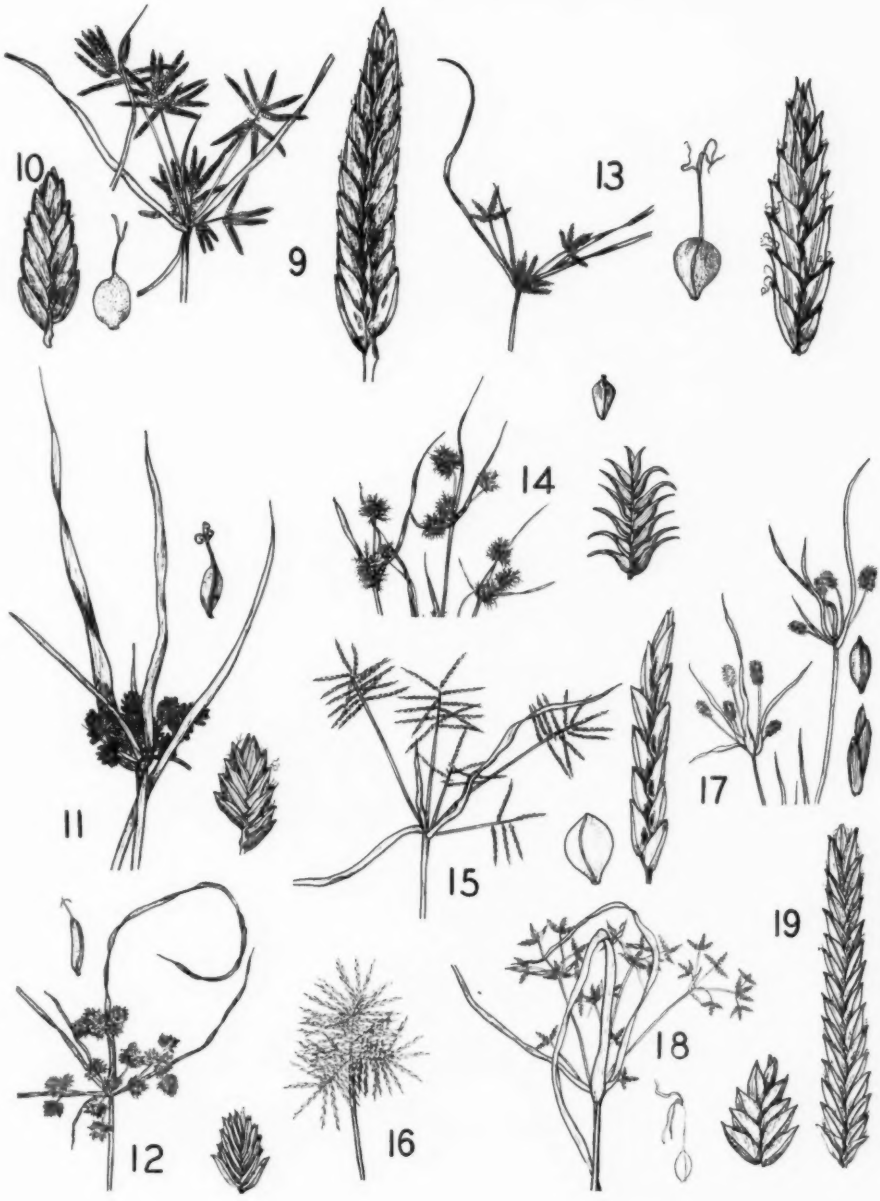


PLATE 27



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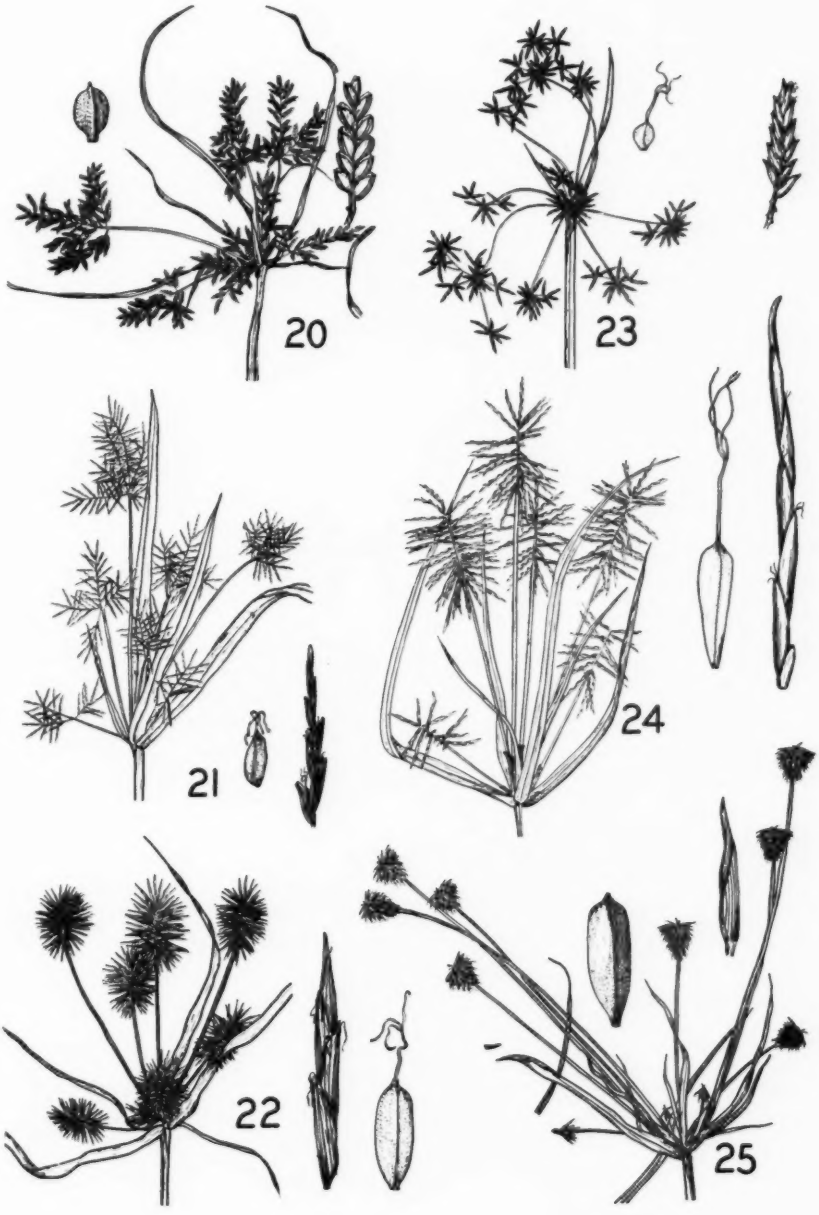

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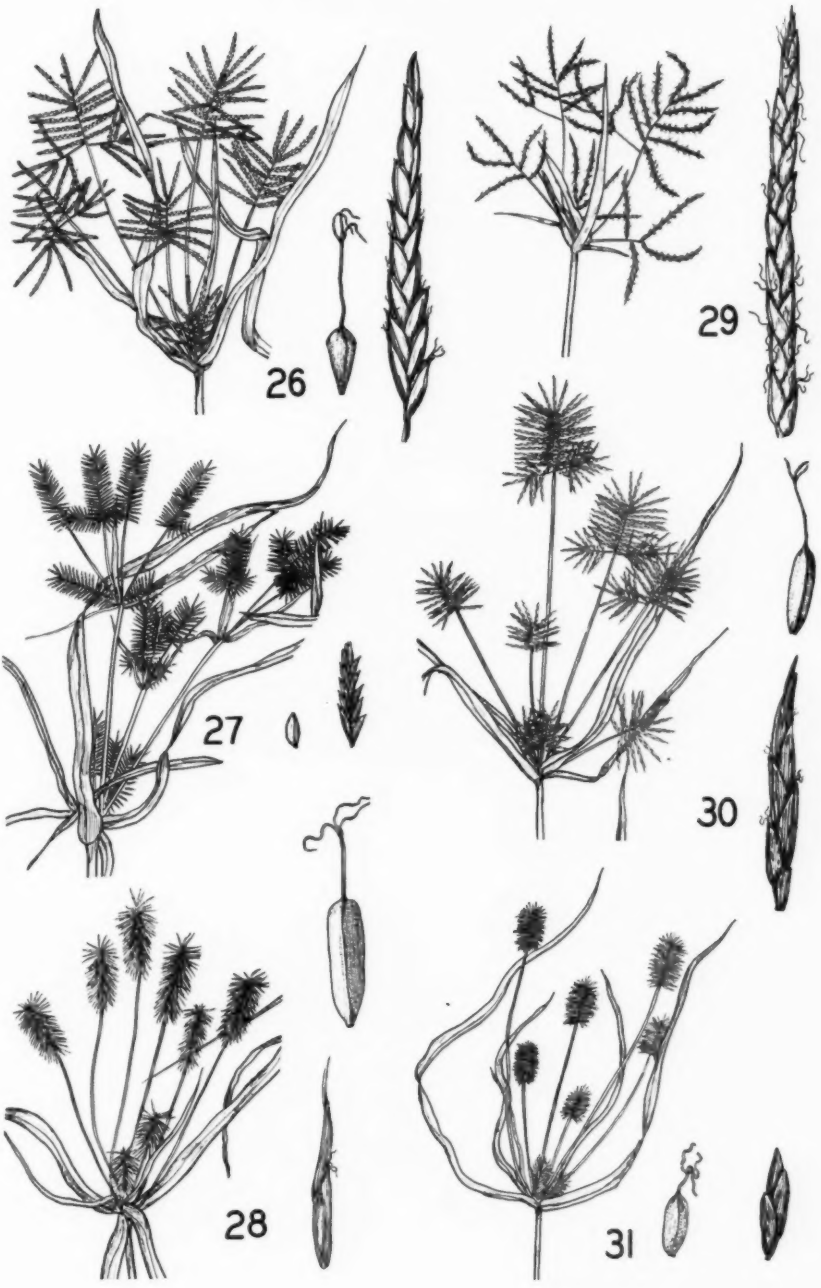
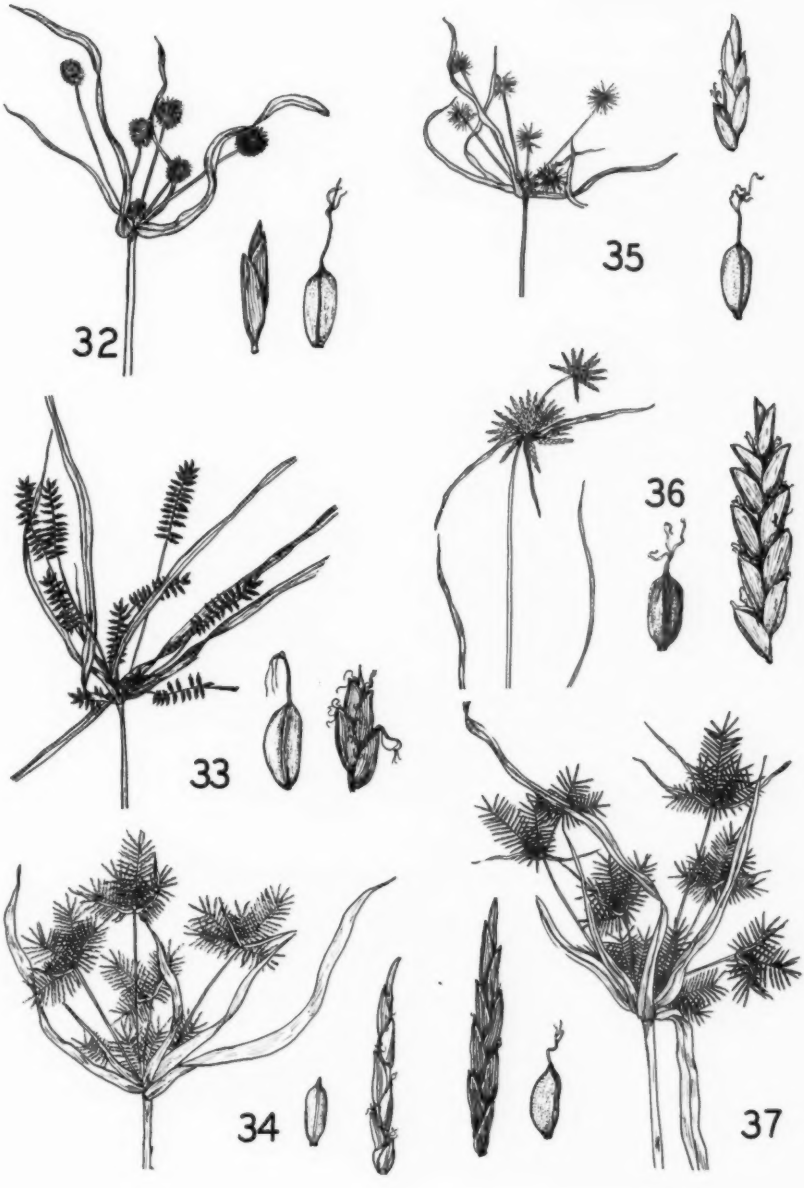


PLATE 30



SPERMATOGENESIS IN MARSILEA

By A. G. Lang

Plates 31–35

INTRODUCTION

Although many detailed studies of spermatogenesis in ferns have been made during the last forty years, most of these investigations have been concerned primarily with the problem of centrosomes and blepharoplasts and until recently no serious efforts were made to interpret the structure of the mature spermatozoids. In 1897 Belajeff (5) announced the presence of a deeply-staining band in the fern spermatozoid and believed this structure to give rise to the flagella in the same way that the blepharoplast of the gymnosperm spermatozoids gives rise to flagella. This interpretation was widely accepted until Mühldorf (28) pointed out in 1930 that the flagella of the typical fern spermatozoid are not attached to the blepharoplast structure but are outgrowths of a plasma band located between the blepharoplast and the nuclear portion of the spermatozoid. Miss Rogers had arrived at essentially similar conclusions four years earlier (31). Shortly after the paper by Mühldorf had appeared, Miss Dracinschi published the results of her investigations (14, 15, 16). She confirmed the observations of Mühldorf and indicated further that a basal granule is found at the insertion point of each flagellum. Miss Dracinschi also believed the blepharoplast structure to be composed of chondriosomal material. Miss Rogers (31) had described similar basal granules sometime previously and Motte (26) had believed the blepharoplasts of the bryophytes and the ferns to be chondriosomal substance. More recently, Yuasa has investigated the morphology of various fern spermatozoids and has largely confirmed the findings of Miss Dracinschi concerning the structural organization of these bodies (46, 47, 48, 50, 51).

Although certain other recent publications, notably those of Weier (41) and Miss Rankin (30), contributed toward the formulation of the present investigation, these need not be discussed here since the following report mainly concerns the structure of the mature spermatozoid. However, the present study was undertaken to trace the occurrence and behavior of all noteworthy cellular constituents during spermato-

genesis and to ascertain more precisely the structure of the *Marsilea* spermatozoid in relation to the reports of Miss Dracinschi, Yuasa, Motte, and Miss Rogers.

MATERIALS AND METHODS

Sporocarps of *Marsilea quadrifolia* were obtained through the kindness of Dr. E. L. Stover at the Eastern Illinois State Teacher's College. Dr. H. C. Aase of the State College of Washington collected sporocarps of *M. vestita*, but unfortunately these were not viable.

For the study of the mature spermatozoid, sporocarps were cut open with a razor blade and placed individually into beakers of water on a hot plate at 28°C. The spermatozoids seem to thrive equally well in distilled water (pH about 5.7) and in tap water (pH about 9 to 10 in Raleigh and about 7.2 in Chapel Hill). At 28 to 30°C., swimming spermatozoids may be obtained regularly in about 6½ hours. They are most abundant usually from 1 to 4 hours after the first dehiscence and the collections for study can be made most profitably about 3 hours after the first swimming spermatozoids are seen. About 2 hours after the first antheridial dehiscence, the inner channel of the mucilaginous envelope becomes suddenly filled with many spermatozoids. Most of these are deprived of their normal vesicle and bear instead a larger and much more hyaline secondary vesicle-like membrane which is sometimes difficult to demonstrate. Embedded in the mucilaginous envelope itself at this time there are found many extended spirals which are especially favorable for study. Material germinated at temperatures lower than 28°C. has shown no differences in any structural detail.

Active spermatozoids were fixed with osmium fumes and stained according to the technics described by Steil (36, 38), Mühldorf (27, 28, 29), Dracinschi (14, 15, 16), and particularly those by Yuasa (47). The most useful preparations of this type were obtained with 1% solutions of iodine green, basic fuchsin, carbol fuchsin, safranin O, and neutral red, but other stains such as acid fuchsin, methyl green, crystal violet, anilin blue, Delafield's and Heidenhain's hematoxylin were also used. Heitz's hot aceto-carmine was most useful when used on material treated for 2 to 5 minutes with strong osmium fumes.

Spermatozoids were also studied from paraffin sections. This material and material of the developmental stages were fixed in the following solutions which were found most useful in the identification and fixation of specific constituents of the spermatozoids and cells of the developmental stages:

1. Sharp's *Marsilea* formula (33).
2. Nawaschin (Craf's modification).
3. Nawaschin (author's modification):

A.	Chromium trioxide	1 gram
	Acetic acid	7 cc.
	Distilled water	92 cc.
		} 13 cc.
B.	4% Formalin	13 cc.
C.	1% Osmium tetroxide	1 cc.

4. Regaud's method, in various forms of post-fixation.
5. Lewitsky's formula (25), used with formalin "neutralized" with $CaCO_3$, or with $MgCO_3$, or used with acid formalin.
6. Lewitsky's formula (author's modification):

A.	1% Chromium trioxide	14 cc.
B.	10% Formalin	14 cc.
C.	1% Osmium tetroxide	0.8 cc.

7. Champy (7-7-4 formula).

Spermatozoids fixed in these and other solutions were stained with various dyes already mentioned. Basic fuchsin, safranin O, iodine-crystal violet, and Heidenhain's hematoxylin were most useful. Formulae 1, 2, 3, 5, and 6 gave best results in the developmental stages of the microspore and Regaud's formula was especially excellent during late spermatoteleosis. Material was washed in tap or distilled water for varying periods of time according to the fixative used and was dehydrated in N-butanol in about 18 steps or it was infiltrated by means of petroleum ether. Acetic acid mordants were especially valuable for hematoxylin staining (23) and the degree of destaining was varied to reduce the probabilities of misinterpretation.

DESCRIPTION

The divisions of the microspore. I have satisfied myself that the cellular development in the microspore occurs precisely as described by Sharp (33). A more detailed analysis of the entire developmental history has not been completed and will not be reported here.

Spermatoteleosis. Being interested largely in the relation between centrosomes and blepharoplasts, Sharp did not attempt to follow the later stages in spermatoteleosis and probably for this reason his description for this phase of development does not agree in all details with my observations.

The first of these details concerns the maturation of the blepharoplast. Following the use of the fixative which Sharp employed, one would clearly be inclined to agree with him that the blepharoplast elongates in the cytoplasm of the spermatid and that during this elon-

gation the blepharoplast extends forward in advance of the metamorphosing nucleus. However, if we study preparations of the Lewitsky or Nawaschin type, we would be inclined to interpret the relation of the blepharoplast to the nucleus somewhat differently. Such preparations would lead one to the interpretation that the blepharoplast, almost from the first phases of its elongation, is located within the nuclear membrane and that it never extends forward beyond this membrane. The blepharoplast elongation would appear, not merely to precede the nuclear elongation, but to be a mechanical agency which is responsible for the elongational distortions of the nucleus. The blepharoplast seems to force the nuclear membrane into a condition of apparent stress and seems to be the cause of the membrane distortions which result in the eventual spiral form. During this reorganization of the spermatid materials, the anterior and growing end of the blepharoplast appears to be invested with a very close-fitting portion of the nuclear membrane. The forward part of this membrane at this stage is apparently entirely devoid of structurally organized contents and could not be seen in preparations where fixatives of the "nuclear" type had been used. Following fixations which are more favorable for the preservation of the cytoplasm, however, the karyolymph contents of this membrane can be seen as a transparent space which is definitely delimited from the dark cytoplasmic background. This nuclear portion is very thin at the extreme anterior end even at maturity. It would seem probable, therefore, that the blepharoplast becomes intra-nuclear at an early stage and that it remains within the nuclear membrane thereafter. Further evidence in favor of this interpretation will be brought forward later.

During the early metamorphosis of the nucleus, the chromatin is organized very much as shown by Sharp (33, Figure 37). This material soon clearly resolves itself into four definite chromonemata which as almost parallel strands make their way forward into the anterior portions of the spiral nuclear membrane. Eventually they are spacially arranged as four parallel chromonemata which extend from the posterior end of the spermatozoid well forward into the anterior end. This chromatin behavior can be followed clearly in preparations stained with iodine-crystal violet following Sharp's fixative.

Concurrently with this nuclear reorganization, the cytoplasm undergoes a remarkable series of changes. Even before the nucleus and the blepharoplast have formed one complete spiral, the distending nuclear portion already lies in contact with the plasma membrane at the periphery of the spermatid. The blepharoplast is at the outer limit of the

nuclear spiral. In the central part of the spermatid, from which the nucleus is being withdrawn, there appear, in contact with the nuclear membrane, a number of large, clear spaces which are interpreted as vacuoles. As the nucleus becomes more completely retired from this central portion of the spermatid, the vacuolated region increases in size and certain materials from the cytoplasm move into this formative vesicle. A number of small but conspicuous starch grains develop here very soon after the vesicle first becomes delimited as a vacuolated region. The large starch grains in the peripheral parts of the microspore concurrently undergo a partial digestion. When the development of the spermatid is proceeding at a temperature of 30°C., the first starch appears within the newly-formed vesicle about fifty minutes after the blepharoplast begins to elongate. The mature vesicle contains within its membrane a loose network of cytoplasmic strands in which are suspended a number of plastid primordia. Some of these plastid bodies remain free of starch. A ribbon-like band of substance of an undetermined nature is also found in the mature vesicle, its component substance having been derived from many scattered, irregular-shaped, and chromatic masses of material which are found in the cytoplasm of all spermatogenous cells. This material is presumably comparable to the heavily-staining material found in the vesicles of various bryophyte spermatozoids by Steil (37).

The cytoplasm at the posterior end of the spermatid becomes progressively thinner as the vesicle increases in volume. The cytoplasm migrates into the anterior half of the spermatid and forms a long, cytoplasmic spiral which is at first thickest at its anterior end. The outer and peripheral side of this cytoplasmic spiral is continuously concave throughout its length. The developing nuclear spiral lies within this concavity and during its development always moves forward in this cytoplasmic channel. When the nuclear coil has become almost complete, the cytoplasmic spiral withdraws slowly to the anterior end where it forms a semi-cylindrical, compact block of cytoplasm which partially surrounds the anterior, free coils of the spermatozoid. In this anterior mass of cytoplasm, by Regaud's technic, are seen several very distinct and intensely chromatic bodies. Sometimes all of this material is found in a single conspicuous spherical body (Figure 16). This is apparently the "Nebenkern" which Yamanouchi described for *Nephrodium* (44).

Cilia can be found growing from the region of the nucleus long before the spiral body is complete. They are entirely unstained when hematoxylin is used. At first they are very thick and short, but as they

grow in length they become very slender. The precise point from which the cilia first develop has not been determined. When fully grown, the cilia are probably less than .1 micron in diameter and bear "end pieces" which are about 4 microns long. The length of the flagella could not be determined accurately because the flagellar filament becomes coiled into a regular spiral which gives one an erroneous impression that the flagella are approximately .18 of a micron wide.

Shortly before antheridial dehiscence, the spermatozoids are symmetrically arranged within each antheridium in two groups of four. The cytoplasmic cap is left behind when the spermatozoids are liberated.

Various other less obvious features of spermatoteleosis will not be described at this time.

The mature spermatozoid. The general appearance of the spermatozoid is indicated by figures 1, 2, 3, 4, 5, 17, 18, 19.

The blepharoplasts. In paraffin material, the most conspicuous structural elements of the spermatozoid are the blepharoplasts. These extend from the anterior tip of the spermatozoid to the posterior end, but occasionally the nuclear body extends several microns beyond the blepharoplasts at the posterior end.

The blepharoplasts are very obviously two parallel strands rather than a single unit (Figure 9). These two blepharoplasts are morphologically identical. Near the anterior end there are frequently seen very definite clear areas in these structures. These hyaline segments are always of uniform size and appearance (Figure 25). They were ignored at first on the assumption that they result from the sectioning process, but that this is not the case can be readily established by means of focusing alone. Further evidence that they are characteristic of the blepharoplasts is found in their constancy of position. In one instance a similar clear segment was seen in only one of the two blepharoplasts. Each blepharoplast is at its widest point about .3 to .4 of a micron in diameter and in its anterior end the diameter is apparently less than one-fourth as great.

These blepharoplasts give some evidence that they have an internal structure. When hot aceto-carmine is applied to the spermatozoids which have been treated with osmium fumes for about five minutes, one can detect a very minute and preferentially-stained coil which seems to be located within a blepharoplast or intimately near it. Only by very delicate focusing can one ascertain the coiled nature of this chromatic substance. This coil is extremely uniform in its windings, but its

coiled nature could be established only in the thicker portions of the blepharoplasts. The substance composing this coil, however, extends over the entire length of the spermatozoid. The aceto-carmine soon becomes general throughout the blepharoplasts and these details become obscure. The blepharoplasts, however, are then revealed as two very obviously separate strands (Figure 9). Material fixed with Lewitsky's solution and stained with Heidenhain's hematoxylin will, under conditions of strong destaining, also indicate the existence of a blepharoplast coil.

In the light of these observations it is interesting but perplexing to consider certain results with vital dyes. Neutral red (1:20,000), when applied to living spermatozoids, will stain the peripheral parts of the blepharoplasts and give these structures the appearance of two hollow tubes. Methyl green, similarly applied, will stain the blepharoplasts in the same manner but less conspicuously. Janus green, under the same conditions, will, in time, stain the parallel blepharoplasts as a single and very slender filament.

These observations would seem to indicate that the blepharoplasts may be two-component structures, both components being essentially similar substance, the one being organized as a matrical system about the other very much as chromosome matrices may enclose within them the chromonemata. This interpretation of an internal structural differentiation in the blepharoplasts, however, needs to be verified in more favorable material.

The spermatozoid body. The body portion of the spermatozoid consists primarily of karyolymph. This part of the spermatozoid is characteristically circular in cross-section, but in many cases the body might become greatly distorted. If we bring into consideration the effects of the various microchemical reagents, we find that the body, at its widest point, is usually about 1.7 microns in diameter. At the anterior end it is approximately one-half micron in diameter. The entire spermatozoid in the swimming condition is a spiral consisting of about seven to nine turns. The blepharoplasts are always seen at the extreme inside of this spiral, but in partially extended spirals the blepharoplasts are usually found at the upper and forward side of the body as shown by figures 10, 11, 13, 19, and by Sharp (33, Figure 42). When fully extended the spermatozoid body is approximately 110 to 120 microns long.

Paraffin material stained with Heidenhain's hematoxylin following Nawaschin and Lewitsky fixations affords perhaps the best view of the

chromatin and its true spacial relations. Other stains and fixatives can be used with excellent results.

Except for the blepharoplasts, the most conspicuous structures of the spermatozoid are the four chromoneme threads. Following certain fixatives these structures appear to be diametrically uniform chromatic filaments which extend almost from end to end in the spermatozoid. Following other fixations, the chromatin filaments are seen as thin, granular threads, that is, as chromonemata. In the widest part of the spermatozoid body, the four chromonemata are almost invariably found to be in two pairs. At times, especially in the thinner parts of the spermatozoid, only two chromatic threads can be found, but these are often more conspicuous. Still further forward, only one chromatic thread can be seen on most occasions. Such observations would seem to indicate that four continuous chromonemata extend, with varying distinctness from each other, over a considerable length of the spermatozoid. They are always peripheral in position and in cross-section views of the spiralled body they are regularly found on the outer and posterior sides showing a tendency to lie in two pairs. They may be seen in osmium-aceto-carmine preparations, but their true spacial relations are less conspicuous by this technic. No discontinuity in the chromonemata has been observed.

Besides the four chromonemata in the karyolymph portion of the spermatozoid, on rare occasions there was seen one other much less conspicuous granular thread. This very delicate filament has been observed only on the blepharoplast side of the spermatozoid body and in one instance was clearly parted into two filaments for a distance of about 8 microns. Although it may be composed of chromatin, this thread is definitely not identical with the chromonemata which may be concurrently in evidence.

No other structural elements were seen in the karyolymph by any technic employed.

The cilia-bearing band. The two blepharoplasts rest in intimate contact with a distinct portion of the body which Yuasa (46) has referred to as the "cilia-bearing band." This band together with the blepharoplasts will be referred to as the *locomotor portion*.

In the case of the *Marsilea* spermatozoid, the cilia-bearing band is quite small and inconspicuous and, perhaps because of this, has been demonstrated by only two technics.

The stains successfully used by Mühldorf, Dracinschi, and Yuasa

have failed to discriminatingly stain this portion of the *Marsilea* spermatozoid. With iodine green, however, when this is used with osmium-treated material, a bi-chromatic staining is easily affected. When such preparations are studied with the most favorable type of heterochromatic yellow-green light, the blepharoplasts and the chromatin-bearing portion appear brownish-red and the remaining part of the spermatozoid body remains blue-green in color. This blue-green portion is the cilia-bearing band and varies in width from about one micron at its widest point to about .4 micron at the anterior end. In the posterior coils it may be seen from either one or both sides, whereas in the anterior coils it is constantly seen from both sides of the body.

This cilia-bearing band and its internal structure can be analysed more successfully by a steaming process which differentially removes various parts of the spermatozoid in a definite sequence according to their nature. A large drop of water containing many free-swimming spermatozoids is placed on a slide which is then put horizontally (right side up) over a beaker of steaming water. At necessary intervals hot water is added to the slide. After five to ten minutes of this treatment the water is allowed to evaporate completely. Sometime after the slide is dry on its upper side, the slide is cooled in water and stained with basic fuchsin. It is then dried quickly and mounted in balsam.

In one such preparation, it is usually possible to find fully extended spermatozoids in almost all stages of disorganization. Some are almost entirely normal in their constituent parts, that is, the blepharoplasts, cilia-bearing band, and the chromatin-bearing portion are all present without noteworthy structural change. Other spermatozoids show a first step in disintegration. This consists in the gradual loss of the karyolymph portion. The karyolymph breaks up into a number of small globules (Figure 20) which frequently can be seen clinging to the flagella. These globules of karyolymph substance move along the flagella away from the spermatozoid body and leave behind the locomotor portion of the spermatozoid. This is composed of the blepharoplasts, the cilia-bearing band, and the flagella (Figure 21). The next phase of disorganization occurs significantly later. This consists of a break-down of the cilia-bearing band. The ground substance of this band passes off in the form of small globules much as the karyolymph becomes removed (Figure 22). After this ground substance has disappeared, there remain two distinct types of structure. The first of these is the pair of blepharoplasts and the second is the flagellar apparatus (Figures 23, 24). At this phase of the break-down, the flagellar

apparatus is seen to consist of two strands of unstaining material. These strands may or may not continue to lie in a position parallel to the blepharoplasts. One of these strands gives rise to the flagella. This *ciliophore*, as it will be designated here, is of the same diameter and is composed of the same substance as the flagella. The second strand is almost identical with this functional ciliophore, but it bears no flagella or other outgrowths. On some occasions it becomes entangled with the functional ciliophore, this being due to the treatment. In the undisturbed spermatozoid these two ciliophores are parallel strands (Figure 19), the non-functional one being the innermost. The non-functional strand is presumably an undeveloped ciliophore, in view of the phylogenetic history of the flagellar apparatus and also in view of the flagellar relations in certain more advanced polypodiaceous spermatozoids where two, three, or four rows of flagella exist in contrast to one row in *Marsilea*.

As the disintegration progresses, the flagella lose their customary form and become globules of flagellar substance (Figure 24). When the disorganization proceeds further, the flagellar globules become larger and the flagella become shorter. The non-functional ciliophore concurrently shows evidence of decomposition over its entire length. The blepharoplasts are the last structures to survive the treatment. No evidence of a fibrillar composition of the flagella has been noted by any of various technics.

On the basis of this morphological analysis, the *Marsilea* spermatozoid consists essentially of the following parts (Figure 25):

(1) The two blepharoplasts.
(2) The cilia-bearing band which is composed of a "ground substance" and two ciliophores, one of which gives rise to the flagella.
(3) The chromatin-bearing portion which is composed of karyolymph and four chromonemata.
(4) The membrane which encloses the entire spermatozoid, exclusive of the flagella.

DISCUSSION

Spermatoteleosis

The cytoplasmic cap. In the literature on spermatogenesis in ferns we find no accurate description of the rôle of the cytoplasm during spermatoteleosis. It seems agreed generally that the cytoplasm of the spermatid becomes diminished in quantity as the metamorphosis progresses and that the cytoplasm is eventually found in the posterior

vesicle. In some instances it is believed that a portion of the cytoplasm contributes to the material of the spermatozoid. In *Marsilea* it is evident that the cytoplasm does not decrease to any noticeable extent during the metamorphosis of the spermatid. The portion of the cytoplasm which contributes to the material of the vesicle represents but a small part of the whole, since the vesicle consists largely of vacuoles and starch grains. The remainder of the cytoplasm resolves itself into the cytoplasmic cap and appears to represent all the remaining cytoplasm.

The spiral cone of cytoplasm with its concavity in which the spermatozoid body lies during the earlier phases of its development was seen by Shaw (35) who also figured it (Plate XI, figure 20).

If we may judge by the figures for *Nephrodium* given by Yamanouchi (44), his figure 32 seems especially to suggest that he saw the cytoplasm on the outside of the spermatozoid spiral in this phase of spermatoteleosis. This seems to be borne out by the position in which he figures the "Nebenkern" for in *Marsilea* a body which compares favorably with his description of the "Nebenkern" is frequently found in this posterior region of the cytoplasmic cone during a corresponding phase of maturation. These and other features of Yamanouchi's description suggest that the *Nephrodium* type of development is essentially identical with that of *Marsilea*. It would therefore seem reasonable to suspect the presence of cytoplasmic caps in all of the polypodiaceous ferns. In *Marsilea* the "Nebenkern" substance is presumably chondriosomal in origin.

It is of particular value to recall at this time that Yuasa has described two "plasma-blocks" associated with the *Isoetes* spermatozoid (49, figure 6). One of these is in the usual posterior position whereas the other is found near the anterior end. This suggests that the posterior one is the vesicle and the anterior one is homologous to the cytoplasmic cap of *Marsilea*. A review of the literature will almost inevitably lead one to the suspicion that the ferns and fern allies probably all produce individual structures which are homologous to the vesicle of *Marsilea* and individual structures which are homologous to the cytoplasmic cap of this form. That homologies of this type may exist throughout the pteridophytes is no more remarkable than the homologies which are known to exist in other features of their spermatozoids or spermatogenous divisions.

The spermatozoid of *Equisetum* does not present an obstacle to this line of reasoning for the vacuolate posterior cytoplasm would be a vesicle

by homology. Even in *Chara* the spermatozoid is found to have a posterior, starch-bearing mass of cytoplasm (Mühldorf, 28). This portion, by homology, would also be a vesicle. It seems obvious that true vesicles are very general in the bryophytes. The presence of cytoplasmic caps in plants other than *Marsilea* (bryophytes, pteridophytes, and certain thallophytes), however probable their existence may seem, remains to be established by further investigations.

The elongating blepharoplasts. The ontogeny of the blepharoplast in the ferns has been studied and described in greater detail than any other single feature of spermatogenesis.

Investigators seem to be of the opinion that the blepharoplast elongates outside the nuclear membrane (44, 1, 32, 33, 30, 50). In some instances the blepharoplast is in close union with the nuclear membrane from the earliest period of elongation (*Nephrodium*, 44; *Marsilea*, 33; *Polypodium* 30). In other instances the blepharoplast undergoes some elongation before it becomes applied to the membrane (*Equisetum*, 32; *Notogramme* and *Pteris*, 50; *Salvinia*, 45). In some forms the blepharoplast is said to develop noticeably in advance of the nuclear membrane (*Marsilea*, 33; *Polypodium*, 30). Weier (41) working with mosses and Miss Rankin (30) working with *Polypodium* have agreed that the blepharoplasts are outside the nuclear membrane during the period of elongation. This is especially significant because both investigators employed cytoplasmic fixatives and technics which should be particularly favorable for the study of this feature. Weier further points out that in *Polytrichum* the two blepharoplasts eventually separate from the nuclear portion of the spermatozoid body and become cilia. It would seem, therefore, that this detail has been disposed of satisfactorily.

The author's observations, when considered entirely apart from the findings of others, would definitely support the interpretation that the blepharoplasts of *Marsilea* are within the nuclear membrane during the greater part of their elongation. During the earliest phases of blepharoplast growth these structures are seen as two rigid and thick bodies within the undeformed spermatid nucleus. As these blepharoplasts grow in length they protrude into the nuclear membrane very much as a pencil would distort an inflated rubber balloon from the inside. By the time one full spiral has developed, the blepharoplasts are closely invested with this difficultly-discernible membrane. When the blepharoplasts are preferentially dissolved from the spermatozoid, it is found, by studying various stages of dissolution, that the ciliophores remain undisturbed within the gamete membrane and in favorable material the membrane can be seen to encompass the partially digested blepharo-

plasts. It would further seem entirely likely that all spermatozoid components are contained within a protective membrane. For these various reasons it is suggested that the *Marsilea* blepharoplasts are intranuclear structures during their elongation and at maturity.

The blepharoplasts in all phases of their existence are distinctly rigid bodies. Spermatozoids fixed with aceto-carmine which swells the component substances and spermatozoids fixed with cytoplasmic fluids strong in formalin which shrinks these components strongly indicate such a rigidity of the blepharoplasts. Hot water causes the spermatozoids to unroll; ostensibly this is due to the straightening of the blepharoplasts. When the blepharoplasts are preferentially dissolved from a spermatozoid, this body either loses its spiral form completely or simply collapses into a flaccid, flattened mass. It would seem reasonably justifiable, then, to regard the blepharoplasts as the form-giving elements of the spermatozoids.

These blepharoplast conditions in *Marsilea*, if they exist in fact, do not necessarily conflict with other interpretations on cytologically more primitive forms. An effort will be made elsewhere to indicate that intra-nuclear blepharoplasts could be interpreted as phylogenetically advanced types.

The Mature Spermatozoid

The major structural features of the spermatozoid as described for *Marsilea* have been reported previously in other plants.

Weier (41) has reported that the blepharoplast of *Polytrichum* is a double structure and has referred to these as two blepharoplasts. In the algae, *Oedogonium* (22) and *Derbesia* (13) are known to form double blepharoplasts comparable to those of *Marsilea* (see also Fritsch, 17). The doubleness of the blepharoplast as reported for *Marsilea* by Sharp (33), if we consider his figure 42, is not the same condition referred to above. In his drawing, the lower strand is almost certainly identical with the two ciliophores described in this paper (compare with figure 19). This interpretation would seem to explain why most investigators have believed the blepharoplasts of the ferns to be cilia-bearing organs.

The existence of two blepharoplasts of the *Marsilea* type in other spermatozoids of the *Pteris* type and including the spermatozoid of *Equisetum* would seem highly probable. A more detailed discussion of double blepharoplasts in algae, fungi, bryophytes, and ferns will be presented in a later paper and an effort will be made to indicate the probable homology and evolutionary history of these structures.

The structural organization of the chromatin in fern spermatozoids

has never been accurately described. Most investigators have believed with Yamanouchi that the chromatin becomes greatly compacted during spermatoteleosis as the spermatid nucleus becomes reduced in volume. Eventually the chromatin is said to become homogeneous and unstaining. Most investigators have generally agreed that the entire spermatozoid consists of a nuclear portion and a blepharoplast from which the cilia originate. Although more recent investigations have shown that the flagella certainly do not arise from the blepharoplast, it is still generally thought that the chromatin of the spermatozoid is greatly compacted, homogeneous, and unstaining or that the chromatin exists as a fairly coarse coil of material in the nuclear portion of the body. We might analyse these interpretations more closely in relation to the conditions found in *Marsilea*.

It seems generally agreed that the spermatid nucleus becomes greatly reduced in volume during its metamorphosis. By direct observation alone, this would seem to be the case. If, however, we make measurements and compute the volume of the spermatid nucleus before spermatoteleosis and compare this volume with the volume of the karyolymph portion of the mature spermatozoid, we find that the reduction in volume is more apparent than real. With critical technics, such measurements can be accurately made. The volume of the spermatid nuclei, before transformations in form become initiated, is approximately 110 μ^3. The volume of the chromatin-bearing portion of the mature spermatozoid is approximately 100 μ^3. If the measurements are accurate to within 10%, which they seem to be, we can see that a contraction of the nuclear portion of the spermatid is less an actual one than a casual observation would lead us to believe. The interpretation that the chromatin becomes greatly compacted in the spermatozoid seems therefore to be an untenable one.

The chromatin in the *Marsilea* spermatozoid exists as four parallel chromonemata which extend in a linear fashion from the posterior end of the spermatozoid well forward into the anterior end. These chromonemata do not form a spring-like coil within the body as described for *Leptogramme* by Yuasa (47). It is true that many spermatozoids appear to have such a nuclear structure as Yuasa describes. The author at first believed such a condition to exist in *Marsilea* (see figures 10, 19). This interpretation was based on preparations of the type studied by Yuasa. In every such case, the "internal spiral" was found to be an optical artifact caused by the flagella which become closely and firmly wound about the spermatozoid body. That no internal coil of chroma-

tin materials exists may be convienently demonstrated during the first five minutes of staining in osmium-aceto-carmine preparations.

In support of the present analysis of chromonemata in *Marsilea*, we will recall that Johnson (21) has described the chromatin of *Plagiochila*, a liverwort, to be in the form of chromoneme threads. The author believes it reasonable, therefore, to suggest that a similar disposition of chromatin may be found to exist in the spermatozoids of other ferns, in bryophytes, and perhaps even in the *Characeae*. Certainly this detail would seem to merit attention in further work on these forms, especially since it is difficult to understand the presence of four chromonemata in the spermatozoid of *Marsilea*, a fern whose haploid chromosome number is eight.

The cilia-bearing band of the fern spermatozoid was first reported by Mühldorf (27). Since that time, others have found similar cilia-bearing bands in other fern spermatozoids (Dracinschi, 14, 15, 16; Yuasa, 46, 47, 48, 50, 51). In order to make fully clear the confusion which this discovery has caused, it seems desirable to trace briefly the history of another type of cilia-bearing band as it occurs in other spermatozoids.

Webber, in October 1897 (39), applied the term "blepharoplast" to the structure in the *Zamia* spermatozoid which gives rise to the cilia. This is often referred to as a cilia-bearing band and is characteristically a deeply-staining spiral band. The term "blepharoplast" by derivation means a cilia-forming organ. In the case of *Zamia* as in other gymnosperms, the blepharoplasts are strictly cilia-forming organs since the cilia seem to sprout directly from the chromatic band which bears this name. Almost concurrently with Webber's discovery of the *Zamia* blepharoplast, Belajeff (5) announced the presence of a similar spiral organ in various ferns. He thought this band gives rise to the flagella and referred to it as a cilia-bearing band or "Nebenkern." In the following years it became generally accepted that the chromatic, spiral bands of the fern spermatozoids are homologous to the blepharoplasts of the gymnosperms. The cilia of the ferns were said to originate from the fern blepharoplasts and this cilia-bearing function was believed to be general for all blepharoplasts. This interpretation was widely accepted until Mühldorf in 1930 pointed out that the cilia of certain fern spermatozoids do not originate from this blepharoplast, but that the cilia are outgrowths of a special band which lies in contact with the blepharoplast.

Miss Dracinschi, working in the same laboratory with Mühldorf,

investigated the morphology of a number of fern spermatozoids (14, 15, 16). In 1930 she reported on *Nephrodium* and various other forms. In this publication she criticized Shaw (35) for his use of the term "blepharoplast" in connection with the ferns (*Marsilea* and *Onoclea*) because, she states, this designation positively ill-fits the structure in question since the flagella are not attached to it. She therefore referred to the blepharoplast as a "Randsaum." At the same time she reported the existence of a basal granule ("Basalkorn") at the base of each flagellum. She also attempted to analyse the nature of the "Randsaum" and reported that silver and osmium impregnation methods give negative (?) results with this organ. She summarized this analysis as follows: "Da am vollständig reifen Spermium Mitochondrien nicht zu finden sind, so ist es nicht ausgeschlossen, dasz dieser Randsaum Mitochondrien körper sein könnte." A stiff, basal sheath or "Stiel" was also reported to be present on every flagellum.

In a later publication (16) Miss Dracinschi again pointed out that the "Randsaum" is chondriosomal in nature. She states here (page 96), "Dieser 'Randsaum' wurde von Belajeff fälschlich als 'cilienbildener Faden' (Blepharoplast) bezeichnet, da er glaubte, dasz von diesem Gebilde die Geiseln ihren Uhrsprung nähmen und daran befestigt seien. Auf Grund früherer Untersuchungen (Dracinschi) gelang es für die *Filicales* Spermien zu beweisen, dasz dieser Randsaum ein Chondriosom ist, da er auf spezielle Chondriosomefärbungen positive reagiert. Der Randsaum von *Equisetum* verhält sich genau wie bei den Filicales Spermien und ist daher auch als ein Chondriosom anzusehen. Der Name 'Blepharoplast' kommt ihm also nicht mehr zu, ..."

The results of her investigations may be briefly summarized as follows: (1) the term "blepharoplast" is not appropriate to this structure, (2) the "Randsaum" is to be regarded as a chondriosome, (3) each flagellum bears at its insertion point a basal granule or "Basalkorn," and (4) the locomotor portion or "Stamm" is of "solider plasmatischer Beschaffenheit" and except for the "Basalkörner" and the "Randsaum" has no other structure. These points will be discussed in detail, since they are of great importance if Miss Dracinschi is correct, but first it seems desirable to review the results of Yuasa's investigations.

Yuasa agrees with Miss Dracinschi in several notable points, but he uses a different terminology. The "randsaum" (blepharoplast) he refers to as the "border-brim." The "Stamm" he refers to as a cytoplasmic "cilia-bearing band." It should be noted that he uses the expression "cilia-bearing band" in a different sense than it was used by

Webber (40) and Chamberlain (12). In 1933, Yuasa (47) suggested, "that the so-called blepharoplast which appears during spermatogenesis probably corresponds to the part that includes both the cilia-bearing band and border-brim." In 1934 he described (50) the ontogenetic history of the blepharoplast and the cilia-bearing band as follows: "One edge of the blepharoplast stains deeply and develops into the border-brim, the remaining portion of the blepharoplast coalesces with the nucleus, but the unstained or faintly stained portion remains between the border-brim and the nucleus. This is the cilia-bearing band, ..."

From these investigations certain facts seem quite clear and are supported by my observations on *Marsilea*. The polypodiaceous type of spermatozoid, exclusive of the vesicle, consists of (1) two blepharoplasts ("Randsaum," "border-brim"), (2) a "cilia-bearing band" ("Stamm"), and (3) a chromatin-bearing portion ("nucleus"). The presence of a basal sheath is also verified, but in living material this is quite flexible. In fixed material, however, the sheath may be very stiff.

Certain observation made by Miss Dracinschi have not been verified. She finds a basal granule at the point of flagellar insertion. Yuasa mentions a "basal swollen point." I have used the technics outlined by Miss Dracinschi to demonstrate these granules but find none. Very often one may see distinctly darkened areas where she finds these granules, but such granule-like features in most instances are almost certainly optical artifacts however excellent the optical system may be. The existence of a functional ciliophore in *Marsilea* as revealed by a process of steaming would seem to provide an adequate denial to the reported occurrence of basal granules.

Miss Drachinschi also states that the term "blepharoplast" is not appropriate to the structure which she renames the "Randsaum" and which Yuasa renames the "border-brim." It would seem, however, that the principle of homology should not be ignored in this matter. It is indubitably clear that the blepharoplasts of the ferns are homologues of the gymnosperm and bryophyte blepharoplasts (Sharp, 32, 33, 34). That the fern blepharoplasts are not directly functional in developing flagella is a condition which does not determine homology. The term "blepharoplast" remains as thoroughly appropriate to those structures in ferns so designated by Sharp as it is when applied to the cilia-bearing structure of the gymnosperm spermatozoid. It would seem, then, that the terms "Randsaum" and "border-brim" may be

regarded as alternate names for an organ which is most appropriately named "blepharoplast." Yuasa has more recently suggested that the cilia-bearing part together with the border-brim might be interpreted as the blepharoplast. Although this suggestion seems thoroughly sound, it introduces at once several new difficulties in terminology. The nature of these difficulties will become more apparent in the following pages.

Another interpretation made by Miss Dracinschi and which the present investigation has failed to substantiate concerns the "cilia-bearing band." Mühldorf (28), Dracinschi (14, 15, 16), Yuasa (47) and others have agreed that the cilia-bearing band is cytoplasmic in nature. They base their interpretations largely upon preparations of the osmium-treated type although other evidence is brought forward. The basis for this identification would seem to be somewhat inadequate. If the component substance of the cilia-bearing band be cytoplasmic in character, then we shall expect ordinarily to obtain certain characteristic differences between this substance and the karyolymph when we use fixing fluids of the usual type, such as those of Nawaschin, Lewitsky, and Benda. But none of these fixations discloses the presence of two such types of substances in the spermatozoid body regardless of the stain or stains used. Yet these fixatives set the cytoplasm in strong contrast with the karyolymph in all spermatogenous cells even in unstained material. Aceto-carmine preparations disclose an equally prominent contrast of these substances. With ordinary angiospermous material this contrast between cytoplasm and nuclear lymph is usually much less pronounced or is distinctly absent. In the spermatogenous cell of *Marsilea*, however, it is easily apparent that the cytoplasm is discolored by the fixing fluid or subsequently stains heavily (Figure 14). These facts would seem to deny the presence of ordinary cytoplasm in the mature spermatozoid. That osmium-treated material affords a differential staining with acid and basic dyes is insufficient evidence that if one component is nuclear substance that the other must be cytoplasmic material. Likewise, it does not necessarily follow from such differential staining that if the cilia-bearing band of the fern spermatozoid stains in the same manner as the anterior end of the bryophyte spermatozoid that the two portions are necessarily composed of the same material.

Yuasa (50) has reported that the blepharoplast itself gives rise to the cilia-bearing band. This develpment has not been observed during the present investigation but various lines of evidence support Yuasa's findings in this matter. However, he assumes that the cilia-bearing

band is therefore cytoplasmic substance. Since the author believes that the blepharoplasts are contained within the nuclear membrane at the time that this cilia-bearing band becomes organized into its final form he would interpret Yuasa's observations to indicate that this band originates within the nuclear membrane. This would make it seem less likely that the cilia-bearing band is composed of cytoplasm. The author's analysis on the nature of the blepharoplasts and the cilia-bearing band has been based on an entirely different line of evidence and is presented in part in the next section.

The Blepharoplasts and the Cilia-Bearing Band

The fundamental nature of the blepharoplast has remained a perplexing problem for almost forty years. The earlier investigators were unable to come into agreement in this matter, largely because of conflicting information which arose out of the inadequate microtechnical and microchemical information of their time. In recent years the literature has become greatly enriched in many respects and we are now able to analyse more exactly many of the problems which at first were highly debatable. This condition applies particularly well to the problem of blepharoplasts and centrosomes.

It seems clear today that the blepharoplasts of the thallophytes, bryophytes, pteridophytes, and gymnosperms are all strictly homologous structures. It would likewise appear obvious that blepharoplasts, in many instances, are ontogenetically derived from centrosomal material. These deductions, however, tell us nothing of the real nature of the blepharoplast. In an attempt to solve this problem, various suggestions have been made.

One suggestion has been more widely accepted than any other. Most investigators have agreed with Webber (40) that the blepharoplast, as a centrosome-like body, is an organ *sui generis* and that it appears and develops in the cytoplasm during the spermatogenous divisions. These investigators hold the view or imply that blepharoplasts are cytoplasmic organs.

Another suggestion found in the literature is that the blepharoplast represents the chondriosomal material of the spermatid (Motte, 26; Dracinschi, 14, 15, 16).

Certain others believe to have adduced evidence that the blepharoplast substance finds its ontogenetic origin within the nucleus (20, 24, 12, 13, 42, 4, 11, and others) and most of these investigators hold to the view that the blepharoplast finds its origin specifically in the nucleolus.

The "Hautschicht" origin which Strasburger proposed has also received frequent comment, but it is doubtful that Strasburger would maintain this view today.

We find therefore, that three outstanding points of view find support in the cytological literature at the present time: (1) the blepharoplast is a cytoplasmic organ *sui generis*, (2) the blepharoplast represents the spermatid chondriome, and (3) the blepharoplast is a nucleolar derivative.

None of these three views has been shown to satisfy all the events of spermatogenesis and none of them has been shown to provide us with a better understanding of the various phenomena which are either ontogenetically or phylogenetically associated with the development of the blepharoplasts. The author believes that we can at the present time establish the true nature of the blepharoplasts and formulate satisfactory answers to the many questions which have been raised in this connection. To accomplish this it appears desirable to inspect the suggestions which have been made concerning the nature of the blepharoplast.

The blepharoplast as an organ sui generis: If we agree that centrosomal blepharoplasts are cytoplasmic structures which originate *de novo* in the cytoplasm, then our search is at an end. This interpretation, however, affords us no explanation of the questions which have been raised in regard to this structure.

The blepharoplast as chondriosomal material: Motte (26) was the first to present a serious analysis in favor of this view, but Miss Dracinschi (14, 16, 17) has subsequently advanced evidence in favor of the same interpretation.

Both investigators believe the blepharoplast substance to be chondriosomal material because it gives a positive reaction to specific chondriosomal technics. It is quite true that the blepharoplasts do stain more intensely following chondriosomal fixations than they do following the "nuclear type" of fixation. It is likewise true that the blepharoplasts of living material stain quite distinctly with Janus green. But we cannot ignore the fact that the blepharoplasts also stain heavily following the usual nuclear fixatives. They stain less heavily with aceto-carmine, but their component substance persists indefinitely even in this quantity of acetic acid without a noteworthy change in form. This behavior is certainly not characteristic of ordinary chondriome substance. Can we, then, be justified in calling this blepharoplast structure a chondriome?

The blepharoplast as a derivative of the nucleolus: That the blepharoplast is derived from the nucleolus has been advocated by various investigators. This interpretation has been seriously criticized in many ways. At the present time the evidence in favor of this interpretation can be classified as being of two types; that evidence which concerns the mature blepharoplasts alone, and that evidence which is available from studies on the ontogenetic and phylogenetic history of the blepharoplasts. Only part of the first of these two types of evidence will be discussed here. Other evidence will be presented in a later paper.

In an earlier section the suggestion was made that the *Marsilea* blepharoplasts are probably mechanically responsible for the spiral form of the spermatozoid. If this be true, we should expect to find the spermatozoid body rigidly spiralled as long as these structures are present and the body rigidity should disappear when the blepharoplasts are selectively removed from the spermatozoid. The question arises, then, as to what solvent will remove these structures without materially affecting the remainder of the body. Since we are interested in determining a relation between blepharoplasts and nucleoli, or the absence of such a relation, we would naturally look for technics which are specific to nucleoli. For this purpose, staining reactions may be of doubtful value, but various differential solubility tests appear to be more reliable.

Two types of "specific" nucleolar technics are available. With the first of these we may remove essentially all material from a meristematic type of protoplast except the nucleolar substance. This may be accomplished by treating living material with hot water (60 to 90°C. for 5 to 30 minutes). By the second type of technic we can selectively remove the nucleolus (43). Only three specific solvents for nucleoli are known (HCl, sodium borate, and sodium formiate) (43). This would strongly suggest that the nucleoli are extremely individualistic structures in their chemical composition and enhances the value of such tests.

If we fix a root-tip (*Allium*, *Vicia*, etc.) with hot water and stain sections with hematoxylin, we find that the cell contents are removed in a very characteristic sequence. Two substances ordinarily remain in such preparations. The first of these is entirely unstained and consists of the "kinoplasmic" substances of the cell. These are the plasma and nuclear membranes and the strands of similar material which connect these membranes with each other and the strands which connect the nuclear membrane with the nucleolus. The second substance which remains in these preparations stains very intensely and consists of the

nucleolar material in its usual form. This nucleolar substance is extremely conspicuous. Such a definite protoplasmic reaction at once suggests its usefulness in studying the *Marsilea* spermatozoid.

With this technic, then, we may undertake an analysis of the *Marsilea* spermatozoid. By treating the living spermatozoids with hot water, as described in an earlier section of this paper, we find that their constituent parts are selectively removed in a definite sequence comparable to that of a root-tip cell. The karyolymph of the spermatozoid is removed first (Figure 20). The karyolymph is removed first in the root-tips also. When the spermatozoid karyolymph is entirely gone, only the locomotor portion remains (Figure 21). Of this portion, the blepharoplasts alone stain heavily, but the cilia-bearing band also takes the stain very much as does the plastin of a root-tip nucleolus. The flagella are entirely unstained just as the kinoplasm of the root-tip cell remains unstained. The cilia-bearing band disintegrates almost at the same time that the blepharoplasts show the first sign of breakdown (Figure 22). Ordinarily the blepharoplasts and the flagellar apparatus remain fairly normal in appearance until after the cilia-bearing band substance has been entirely removed (Figure 24). The blepharoplasts stain very heavily whereas the flagellar materials are ordinarily unstained. It would seem, then, that in this test we have a definite indication that the blepharoplasts and the cilia-bearing band may be composed of substances which are remarkably similar to the constituent substances of a typical higher plant nucleolus. By this technic, the blepharoplasts would seem to be comparable in their substance to the nucleolin of a higher plant nucleolus; the cilia-bearing band substance would seem to be comparable to the plastin of the higher plant nucleolus; and the component material of the flagellar structures (the ciliophores and the flagella) would appear to be comparable to the kinoplasm of a root-tip cell. From this type of test, it would appear that Strasburger's theory of kinoplasmic cilia is perhaps more significant than we have realized.

We may approach the problem of blepharoplast identification in another way, that is, we may treat the spermatozoids with specific nucleolar solvents.

Two specific nucleolar solvents were employed. One of these was an 8% solution of HCl (of conc't.) and the second was a saturated aqueous solution of sodium borate. Living spermatozoids were placed into separate vials of these solvents for periods of time varying from two to forty-eight hours and at a temperature of 30°C. Following this treat-

ment, the spermatozoids were stained with 1% basic fuchsin. The briefer treatment with these reagents may in a few cases effect only a partial removal of the blepharoplasts, but a longer treatment, as indicated above, removes these structures entirely (Figures 7, 12). The ground substance of the cilia-bearing band dissolves in advance of the blepharoplasts. The ciliophores appear to be undisturbed even after the blepharoplasts have become removed. When the solvent action is essentially complete, it can be seen that the spermatozoid membrane encloses the ciliophores and the chromonemata which may be clearly evident. The spermatozoid membrane can also be seen to enclose the blepharoplasts when these structures are partially dissolved. The most conspicuous feature of the spermatozoids treated in this manner is that they become flattened on the slide and are comparatively shapeless structures. It seems quite clear, then, that the blepharoplasts are responsible for the shape of the normal, mature spermatozoid. It is also evident that preparations of this kind substantiate the view that the nuclear membrane of the mature spermatozoid surrounds the blepharoplasts and the cilia-bearing band just as the developmental stages seem to indicate. This interpretation would also seem to be consistent with the observation made by Yuasa who reported that the blepharoplast itself gives rise to the substance of the cilia-bearing band.

It is particularly interesting that these solvent chemicals which are specific in their action on typical nucleoli of higher plants are also specific in their action on certain spermatozoid constituents, viz., the blepharoplasts and the cilia-bearing band. Since only three reasonably specific solvents for nucleoli are known, it seems quite probable that the spermatozoid constituents in question are composed of substances which are essentially identical with the component substances of ordinary nucleoli. Hot water fixations strongly support this possibility.

From the preceding account we may establish a basis on which we can more logically interpret the structure of the mature spermatozoid. This interpretation is the only one which the author has been able to devise which will reconcile all the observational data available from the literature and from the present investigation: It may be summarized as follows:

(1) The *Marsilea* blepharoplasts elongate within the nuclear membrane and are responsible for the transformation in form which the nucleus undergoes during spermatoteleosis. The blepharoplasts give the mature spermatozoids their characteristic spiral form.

(2) The entire spermatozoid is a complete unit of nuclear substance and contains no cytoplasm.

(3) The blepharoplasts are composed of a substance which in the higher plant nucleolus has been referred to as "nucleolin."

(4) The cilia-bearing band of the spermatozoid is composed largely of the same substance which in the higher plant nucleolus has been referred to as "plastin."

(5) The chromatin exists in the form of four linear chromonemata.

(6) The karyolymph of the spermatid nucleus is present in the fully mature spermatozoid without an appreciable decrease in quantity.

(7) The cilia are kinoplasmic structures that originate from one of two ciliophores located within the nucleolar material of the spermatozoid. There are no basal granules in the *Marsilea* spermatozoid.

The evidence in favor of nucleolar blepharoplasts is by no means exhausted with this type of analysis. We might recall in favor of this interpretation that only one normal cell constituent, the nucleolus, has the property of fragmenting into bead-like chains and spirals similar to those of growing blepharoplasts. This nucleolar feature has been described and illustrated by Zirkle (52) (Plates 5 and 6, Figures 25, 26, 33a, b, 34a, b). Only one normal cell constituent, the nucleolus, is a two-component structure whose components react toward ordinary fixatives and stains as do the blepharoplasts and the cilia-bearing band of the mature spermatozoid.

That the entire spermatozoid consists of essentially nuclear material is also indicated by the report on fertilization in *Nephrodium* made by Yamanouchi (44) who observed an entire spermatozoid, including the flagella, within the egg nucleus during fertilization.

An effort will be made in a later paper to trace the probable evolutionary history of the blepharoplast and to bring forward other lines of evidence which support the notion of nucleolar blepharoplasts independently of the facts given here.

From the foregoing account, however, it would seem highly probable that the spermatozoid of *Marsilea* consists essentially of nuclear material, the predominate constituent substances being five distinguishable kinds: chromatin, karyolymph, kinoplasm, nucleolin, and plastin.

SUMMARY

(1) A study of spermatogenesis in *Marsilea* has been made and a detailed analysis of the mature spermatozoid is presented. The major events of spermatoteleosis are also described.

(2) During spermatoteleosis, most of the spermatid cytoplasm be-

comes reorganized into a cytoplasmic cap without an appreciable decrease in volume. This cap separates from the spermatozoid during antheridial dehiscence. The blepharoplasts elongate within the spermatid nucleus and are responsible for the final form of the spermatozoid. The karyolymph of the spermatid nucleus does not become appreciably reduced in volume during spermatoteleosis.

(3) The mature spermatozoid consists of at least five types of structurally organized substances: chromatin, karyolymph, kinoplasm, nucleolin, and plastin. Since these materials are characteristically found within the nucleus, we may regard the mature spermatozoid as being entirely nuclear in composition. The swimming spermatozoid, except for its vesicle, being devoid of cytoplasm and bearing a complete complement of nuclear materials, is then, a motile nucleus.

(4) The chromatin exists as four linear, parallel, and peripherally placed chromonemata which extend throughout the length of the spermatozoid.

(5) Most of the spermatozoid body consists of homogeneous and unstaining karyolymph.

(6) The flagella are attached to the spermatozoid body on its forward side and arise from one of two parallel strands of flagellar material located within the cilia-bearing band. These two ciliophores extend over the entire length of the spermatozoid. The substance of the flagellar apparatus is kinoplasm and there are no basal granules in the spermatozoid.

(7) A cilia-bearing band rests in close association with the blepharoplasts and consists of plastin which is a prominent component of ordinary nucleoli.

(8) The blepharoplast structure is not a single, chromatic rod of substance, but is composed of two identical and parallel strands of material. The blepharoplast substance is similar to nucleolin of ordinary intranuclear nucleoli.

(9) The spermatozoid structures, exclusive of the flagella, are invested by a single membrane, this being the membrane of the former spermatid nucleus.

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the essential features of the problem and who has given the benefit of his counsel, is deserving of especial gratitude for his assistance.

DEPARTMENT OF BOTANY,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL AND RALEIGH, N. C.

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DESCRIPTION OF PLATES

All photomicrographs were made with a Spencer microscope equipped with a 1.25 N. A. Abbe condenser and a 1.8 mm., N. A. 1.25, achromatic objective in combination with a Leitz "Periplan" ocular (12×), using full numerical aperture.

PLATE 31

Fig. 1. Spermatozoid stained with carbol fuchsin and showing "end pieces" on the flagella. ×1000.

Fig. 2. Partially extended spermatozoid. Stained with carbol fuchsin. ×1250.

Fig. 3. A swimming spermatozoid deprived of its vesicle. Stained with safranin O. ×1250.

Fig. 4. An extended spiral almost detached from the vesicle. Carbol fuchsin. ×1000.

Fig. 5. Two swimming spermatozoids as seen shortly before fertilization. The large, hyaline, secondary vesicles do not show. Fixed with osmium fumes and stained with safranin O. ×2300.

Fig. 6. Swimming spermatozoids like those of figure 5. Photographed to show the coiled blepharoplasts. Safranin O. ×1250.

Fig. 7. A group of about twenty spermatozoids like those of figure 6 after treatment with HCl. Stained with basic fuchsin. ×1375.

PLATE 32

Fig. 8. Spermatozoid fixed with osmium fumes and stained with aceto-carmine. *a*, blepharoplasts. *b*, chromonemata. ×2300.

Fig. 9. Spermatozoid; osmic fixation and aceto-carmine staining. *a*, blepharoplasts. *b*, chromonemata. ×2300.

Fig. 10. Spermatozoid; osmium fumes and safranin O. *a*, blepharoplasts. *b*, ciliophores. *c*, flagella wound about body. ×1250.

Fig. 11. Spermatozoid fixed with osmium fumes and stained with neutral red. Flagella seen in negative view. ×2100.

Fig. 12. Spermatozoid treated with HCl; basic fuchsin. The blepharoplasts and the cilia-bearing band are removed. The chromonemata and the ciliophores remain in the body. ×1500.

Fig. 13. Spermatozoids fixed with osmium fumes; iodine green. *a*, blepharoplasts. *b*, cilia-bearing band. ×2000.

Fig. 14. Sub-median section through a microspore about 15 minutes before dehiscence. *a*, microspore wall. *b*, cytoplasmic cap. *c*, vesicle. *d*, blepharoplasts. Regaud's fixative and Heidenhain's hematoxylin. ×1150.

Fig. 15. Spermatozoids at the time of dehiscence. *a*, cytoplasmic cap. *b*, blepharoplasts. *c*, "Nebenkern." Regaud's fixative and hematoxylin. ×1150.

Fig. 16. Spermatozoid shortly before dehiscence. *a*, cytoplasmic cap. *b*, blepharoplasts. *c*, "Nebenkern." *d*, vesicle. Regaud's hematoxylin technic. ×2225.

PLATE 33

Fig. 17. Spermatozoid partly separated from the vesicle. *a*, vesicle. *b*, spermatozoid body. *c*, "end pieces" of the flagella. Osmium fumes and carbol fuchsin. ×2300.

Fig. 18. Spermatozoid after losing its vesicle. Osmium fumes and basic fuchsin. ×2000.

Fig. 19. Spermatozoid. Compare with figure 10 which has a greater depth of focus. *a*, blepharoplasts. *b*, ciliophores. *c*, flagella wound about body. Osmium fumes and safranin O. ×2000.

PLATE 34

Fig. 20. Spermatozoid treated with hot water. Karyolymph being removed in the form of small globules. Basic fuchsin. ×1375.

Fig. 21. Spermatozoid after the karyolymph is entirely removed. The body consists of blepharoplasts and the cilia-bearing band from which the flagella protrude. Basic fuchsin following hot water treatment. ×2200.

Fig. 22. Spermatozoid treated with hot water showing a slightly more advanced stage of break-down. The ground substance of the cilia-bearing band passing off as small globules. Basic fuchsin. ×1525.

Fig. 23. Spermatozoid treated with hot water, showing the remaining substance following the removal of the ground substance of the cilia-bearing band. ×1375.

Fig. 24. A late stage of break-down of a spermatozoid. *a*, blepharoplasts. *b*, functional ciliophore. *c*, partially dissolved flagellum. *d*, non-functional ciliophore, partly decomposed. Hot water treatment and basic fuchsin. ×1375.

PLATE 35

Fig. 25. A portion of a spermatozoid showing the major constituents. The two blepharoplasts are at the top. In the forward half of the spermatozoid, these may have occasional hyaline segments as shown. The ground substance of the cilia-bearing band lies in contact with the blepharoplasts and contains the two ciliophores. One of these bears flagella over the entire length of the spermatozoid. Four chromonemata are peripherally located in the karyolymph portion. The membrane of the former spermatid nucleus encloses all structures and forms the basal sheaths of the flagella.

PLATE 31

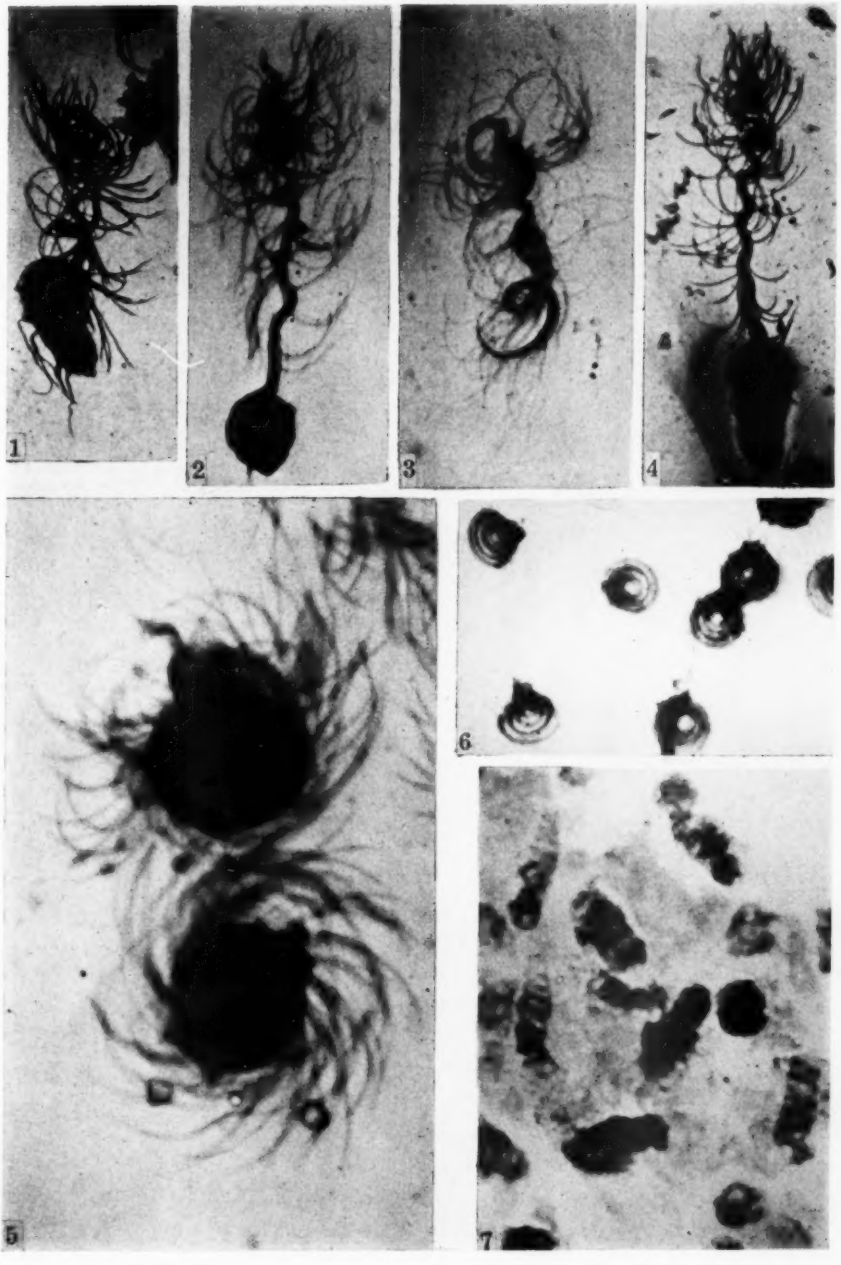


PLATE 32

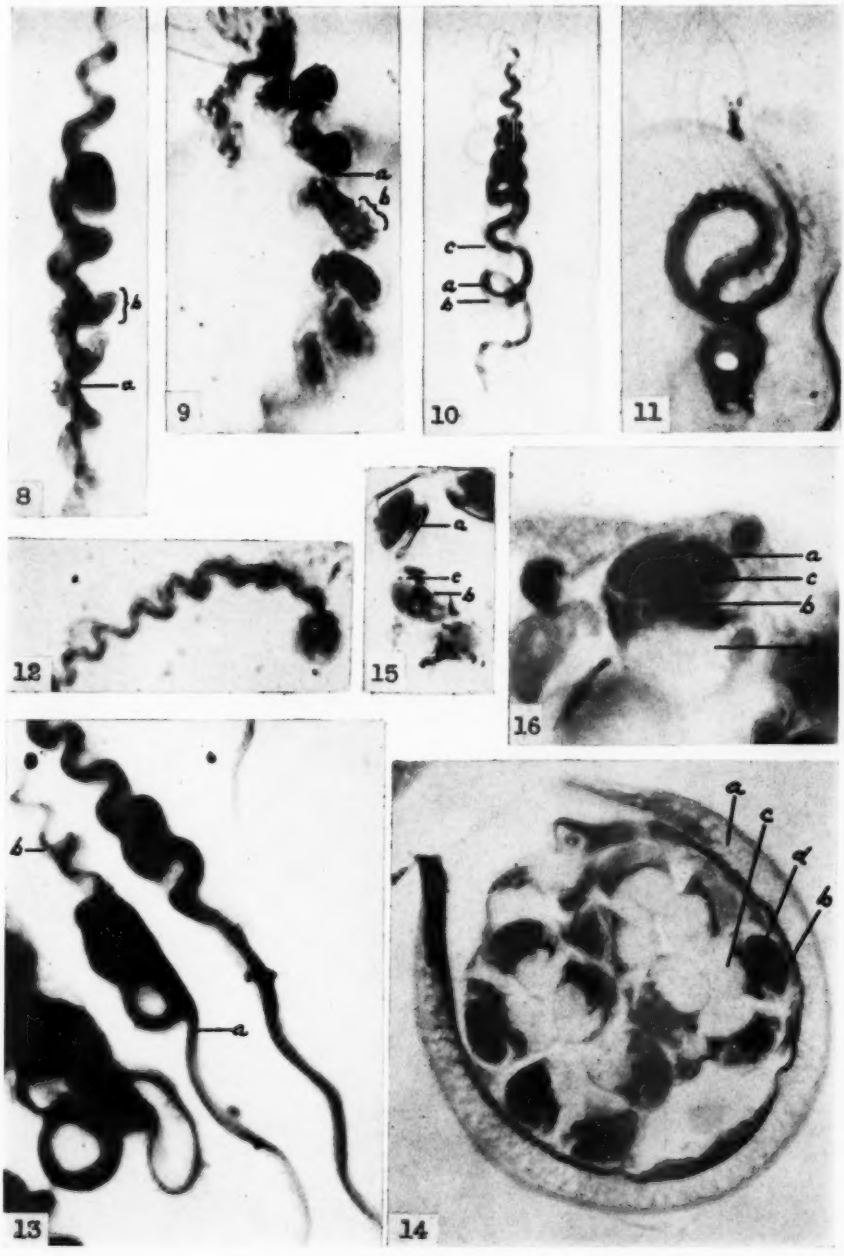


PLATE 33

PLATE 34

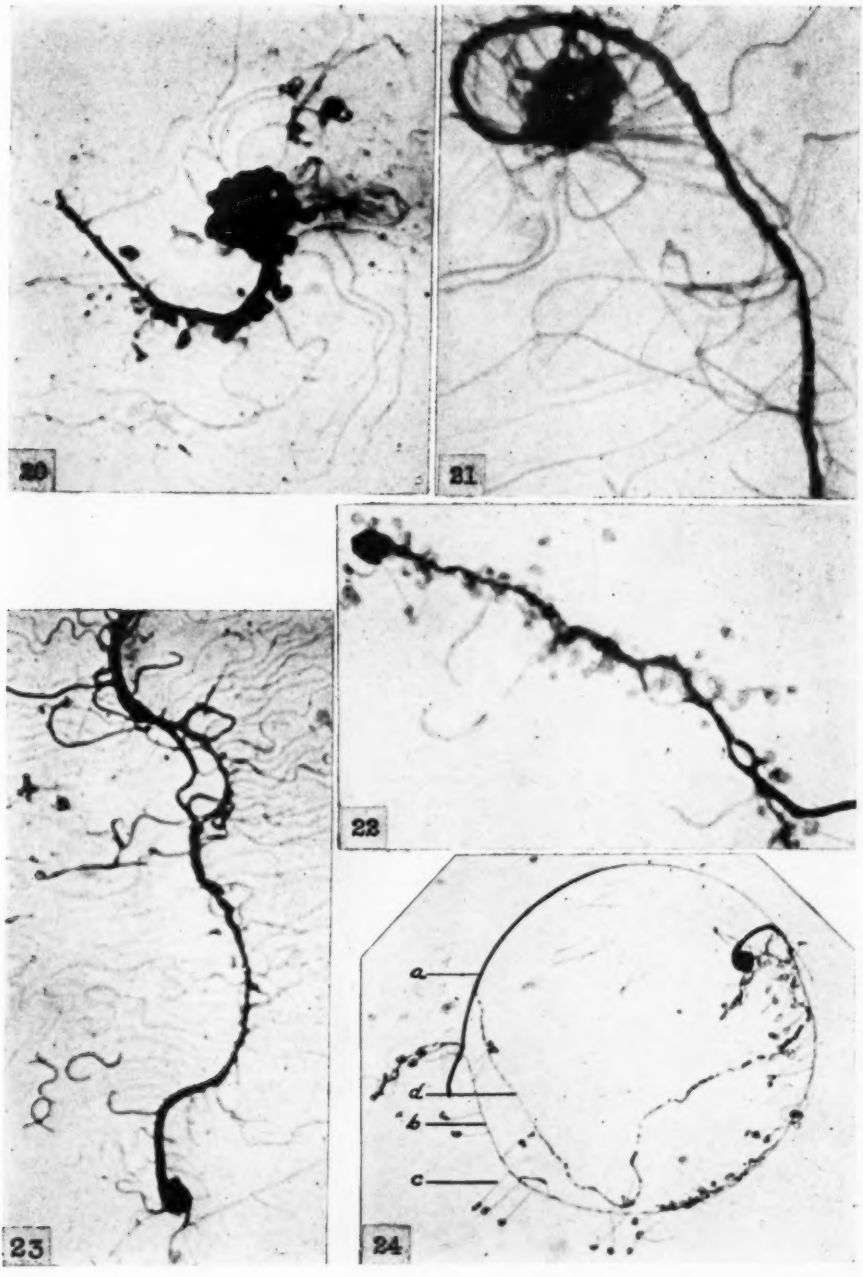
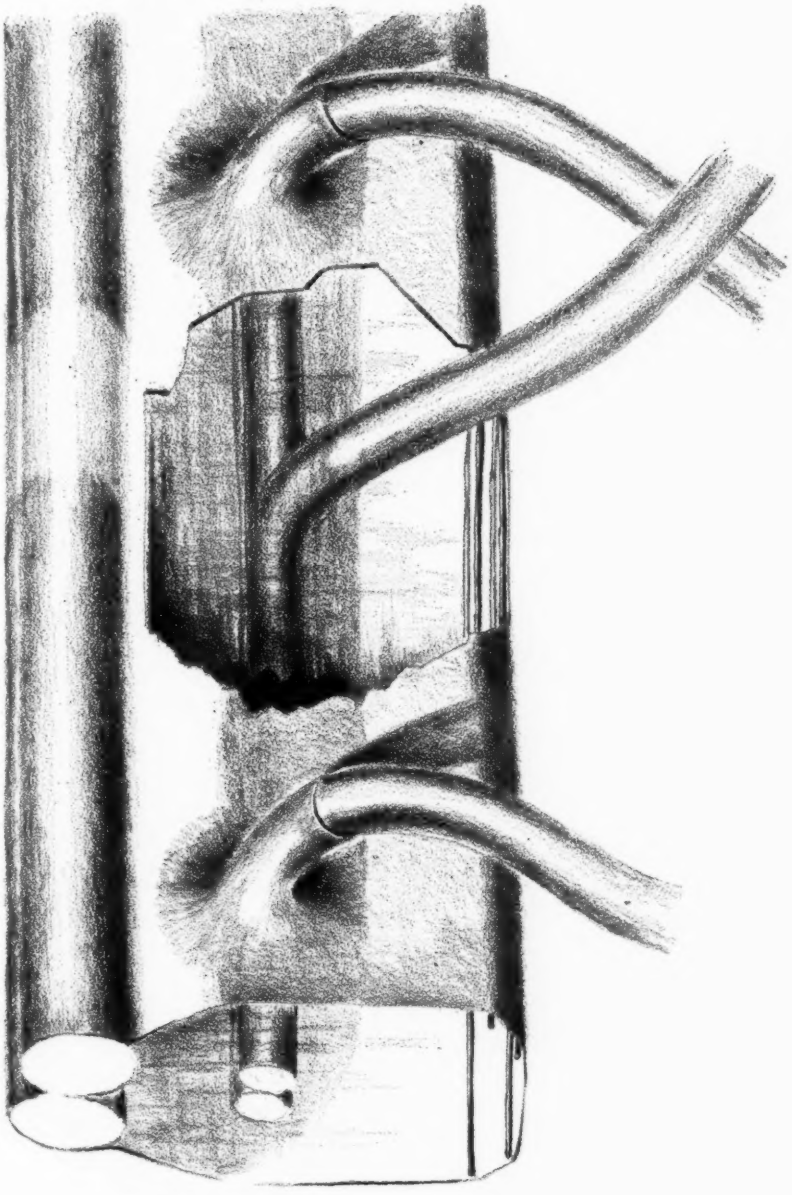


PLATE 35



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CONTENTS OF VOLUME 52

NEW ASPECTS OF RELATIVITY. *Archibald Henderson*..... 1
SOME PRIMITIVE MOSS-MITES OF NORTH CAROLINA. *Arthur Paul Jacot*.... 20
A NEW MULTIPLE CONSTANT TEMPERATURE APPARATUS FOR EXPERIMENTAL WORK IN BIOLOGY *R. E. Coker* and *E. W. Constable*..... 27
RECENT FORAMINIFERA. FROM NEAR BEAUFORT, NORTH CAROLINA. *Wade H. Hadley, Jr*..... 35
THE EFFECTS OF ENDOCRINES ON THE DEVELOPMENTAL RATES OF FRESH FLIES. *E. C. Hester* and *Bert Cunningham*..... 38
THE PHOTOCHEMICAL DECOMPOSITION OF CARBON DISULFIDE. *P. M. Nicholes, N. L. Simmons*, and *H. D. Crockford*..... 51
THE STRUCTURE AND DEVELOPMENT OF THE SEED OF PAULOWNIA TOMENTOSA. *Vera Millsaps*..... 56
A FIELD TRIP TO THE DEVIL'S MILLHOPPER. *Lillian E. Arnold*..... 77
EPIDENDRUM CONOPSEUM IN NORTH CAROLINA. *Donovan S. Correll*..... 91
NEW AND LITTLE KNOWN ALGAE FROM NORTH CAROLINA. *L. A. Whitford*.. 93
THE PRODUCTION OF MATURE PERITHECIA OF CORDYCEPS MILITARIS (LINN.) LINK IN LABORATORY CULTURE. *Leland Shanor*..... 99
A NEW AMANITA AND NOTES ON BOLETUS SUBALBELLUS. *H. C. Beardslee*. 105
THE PSAMMOCHARIDAE OR SPIDER WASPS OF NORTH CAROLINA. *C. S. Brimley*. 107
PROCEEDINGS OF THE THIRTY-FIFTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE..... 133
PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY..... 167
ESTUARINE ANIMALS AT BEAUFORD, NORTH CAROLINA. *A. S. Pearse*..... 174
RACIATION IN STENIRIDIA ANDREWSI HARRIS, A SUPPLEMENT TO SPECIATION IN STENIRIDIA. *J. M. Valentine*..... 223
NOTES ON THE HISTORY OF THE GERM CELLS IN THE TOADFISH (OPSANUS TAU). *Eugene P. Odum*..... 235
MORE PRIMITIVE MOSS-MITES OF NORTH CAROLINA. *Arthur Paul Jacot*.... 247
ORIBATA AND BELBA FROM FLORIDA (ACARINA, ORIBATINAE). *J. W. Wilson*. 255
THE INCUBATION OF HEN EGGS UNDER INCREASED ATMOSPHERIC PRESSURE. *Bert Cunningham*..... 269
HETEROTHALLISM AND STERILITY IN ACHLYA AND OBSERVATIONS ON THE CYTOLOGY OF ACHLYA BISEXUALIS. *John Robert Raper*..... 274
A NEW SPECIES OF RHIPIDIUM FROM MOUNTAIN LAKE, VIRGINIA. *Velma D. Matthews*..... 291
THE GENUS CYPERUS IN NORTH CAROLINA. *Mildred Stites Reed*..... 295
SPERMATOGENESIS IN MARSILEA. *A. G. Lang*..... 307

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